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INVESTIGATIONS ON CERTAIN ESCHERICHIA COLI SERO-TYPES

WITH SPECIAL REFERENCE TO INFANTILE DIARRHOEA

BY

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TURKU . UUDEN AURAN OSAKEYHTIÖN KIRJAPAINO . 1954

To My Wife and Children

PREFACE

The practical work of the present investigation has been conducted at the Department of Bacteriology and Serology of the University of Turku. The Head of the Department, Prof. E. Mustakallio, has through his active interest and encouragement and compliance with my expressed and unexpressed wishes greatly facilitated my work. It is a pleasure to be able to express my sincere gratitude to him.

I am deeply indebted to Prof. T. Salmi, the Head of the Children's Clinic of the University of Turku, for the major part of the case material utilised in the present work, and for invaluable advice and instructive discussions.

For the fruitful cooperation with the Epidemic Hospital of Turku, I am grateful to its Chief, Dr. J. Wikström, University Lecturer.

As in a work of this nature it is imperative that the identification of strains can be based on a comparison with international type strains, I have been fortunate in being able to turn to Dr. F. Kauffmann, the Chief of the International Salmonella and Escherichia Centre, Copenhagen. I wish to acknowledge his kind assistance with many thanks. To Dr. F. Ørskov, of the same Centre, who has also supplied me with types for comparison purposes, and who has confirmed the identification of the *Escherichia* type 55:B5:4, I also extend my thanks. Dr. Joan Taylor, of the Central Public Health Laboratory, London, has also sent me type strains. Her donations have been of great value in my work and for this aid I owe her a debt of gratitude.

I am extremely grateful to Dr. R. Pätiälä, University Lecturer, who has always been ready to enter into discussions which have done much to simplify my problems.

To all my colleagues, who have in different ways facilitated my work, I tender my sincere thanks.

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Turku, June 1954.

J. A. Grönroos.

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INTRODUCTION

The aetiology of infantile diarrhoea is a problem that has been intensively studied during the present century. At the end of the last century and during the first decades of the present century the opinion presented by Czerny (1925) and his school was generally accepted. According to this view, the major causes of infantile diarrhoea were to be found in the dietary errors frequently made at that time and in the readily diagnosed digestive disturbances. Actually at that time a bacterial aetiology could only infrequently be established.

In addition to *Salmonellae, Shigellae* (Hormaeche *et al.* 1943), *Enterococci* (Gale 1944) and *Staphylococci* (Felsen and Wolarsky 1942), also *Escherichia coli* bacteria have been mentioned as aetiological factors in infantile diarrhoea. Already Escherich, who isolated the *Bacillus coli,* suggested such a possibility in 1899. Many investigators, among whom Moro (1916), Bessau and Bossert (1919 *a, b*) and Adam (1923, 1927) may be mentioned, considered the *Escherichia coli* bacillus one of the notable causative organisms of infantile diarrhoea. They were not, however, able to support their opinion with conclusive evidence, primarily owing to the fact that the antigenic structures of the *Escherichia coli* bacilli were not known at that time and that solely on the basis of the biochemical reactions of the *Escherichia* bacilli, it was not possible for the investigators to verify each other's results.

During the 1940's, Kauffmann and his co-workers succeeded in clarifying the serological properties of *Escherichia coli* strains and were then able to classify the *Escherichia* bacilli into clearly defined types on the basis of their antigenic structures. In the light of this development, it is now easy to understand the discordant results of the earlier investigators. This development

naturally led at the end of fourth decade to a reinvestigation of the possibility that *Escherichia* bacilli are aetiological factors in infantile diarrhoea. This work was first begun in England where the views of Czerny and his school had never found favour, and later spread to Scandinavia, Western and Southern Europe and the United States.

A preliminary study conducted by the author early in 1950 revealed that the *E.coli* sero-type 111:B4 frequently isolated from cases of infantile diarrhoea also occurs in Finland (Grönroos 1951 a). Before this, only a few studies had been conducted in which the newest developments in *E.coli* identification had been utilised and differences of opinion existed as to the aetiological significance of *E.coli* in infantile diarrhoea.

The purpose of the present investigation, which is a continuation of the earlier study, has been to obtain additional knowledge of the role played by *E.coli* types as causative agents in infantile diarrhoea. The study has been limited to cover the incidence of the *E.coli* sero-types 111:B4, 55:B5, 26:B6, 44:K?, E 611 and Canioni in material collected in the Turku area.

The main objects of the present investigation have been:

1. *To determine the incidence of the above-mentioned Escherichia sero-types in infantile diarrhoea.*
2. *To study the serological and bacteriological properties of these types and their ability to produce antibodies.*
3. *To pay attention to epidemiological and clinical aspects of infantile diarrhoea in the light of the bacteriological and serological findings.*

I. PREVIOUS INVESTIGATIONS

INVESTIGATIONS CONCERNING THE AETIOLOGICAL SIGNIFICANCE OF ESCHERICHIA COLI TYPES IN INFANTILE DIARRHOEA

Studies conducted prior to the elucidation of the serology of the Escherichia group. — Jensen (1913) was probably the first to conduct a bacteriological study of the significance of *Escherichia* bacilli in diarrhoeal conditions. Actually, his investigations, which were carried out at the end of the 19th century, dealt with the aetiology of white scours in calves. He found that *Escherichia* bacilli of a certain fermentation (A) type were frequently present in the faeces of diseased calves, but were absent from the faeces of healthy calves. When milk infected with A-type *Escherichia* bacilli was fed to calves, the latter developed white scours within five days. The fermentation type B isolated from the faeces of healthy calves was unable to effect symptoms of disease in other calves. Bahr and Thomsen (1912) established that the *Escherichia* bacilli isolated from cases of infantile diarrhoea were of the A type in view of their biochemical reactions. Christiansen (1917) studied the A and B types of Jensen using serological methods. He found, however, that only calves could be used as experimental animals to differentiate between pathogenic *Escherichia* strains causing diarrhoea and apathogenic *Escherichia* strains; neither biological nor serological methods could be employed to distinguish the strains.

On the other hand, other observations had led investigators to assume some connection between *Escherichia* bacilli and infantile diarrhoeal conditions. Moro (1916) had noted that *Escherichia* bacilli are more frequently found in the stomachs and small intestines of children suffering from diarrhoea than in non-diarrhoeal children. These findings were confirmed by several investigators (Bessau and Bossert 1919 *a*, *b* Scheer 1920, Bessau *et al.* 1921 *a*, *b*,

Kramár 1922, Deak 1933, Blacklock *et al.* 1937). Adam (1923) observed that the *Escherichia* strains isolated at autopsy from the small intestines of diarrhoeal infants strongly fermented sucrose. When he compared the isolated sucrose-positive *Escherichia* strains with strains of the fermentation types described by Jensen (1913) and Christiansen (1917), he (1927) established that the former had many reactions in common with the A I and A IV types defined by Christiansen. Goldschmidt (1933) verified Adam's results and was able to establish the relationship between the strains by means of serological procedures. In view of our present knowledge of the serological properties of the *Escherichia* group, Goldschmidt's results must be considered fortuitous. Kyrki (1936, 1944) and Tagawa (1938) were unable to confirm the results of Adam and Goldschmidt, because no reliable method had yet been developed for identifying *Escherichia* strains. In contrast to the latter investigators, Cziglany (1941) reported that he had isolated the so-called *dyspepsia coli* of Adam several times from cases of infantile diarrhoea.

Studies carried out after the elucidation of the serology of the Escherichia group. — When studying a diarrhoea epidemic in 1944, Beavan detected a strong odour of sperm in faeces which was traced to the presence of antigenically homogeneous strains of *E.coli* by Bray (1945). On the basis of their fermenting properties, these strains belonged to the group *Escherichia coli var. neapolitanum*. Bray was further able to show that the strains he had isolated were serologically related to one (N.C.T.C. 198) of the four type strains of *E.coli var. neapolitanum* from the National Collection of Type Cultures, Colindale, London, but differed from the three other type strains.

Giles and Sangster (1948) established the presence of *E.coli var. neapolitanum* in 96 per cent of the diarrhoea cases in an epidemic that spread in Aberdeen in the Spring of 1947. In their studies they were able to take advantage for the first time of the work of Kauffmann and his co-workers on the serology of the *Escherichia group*. We shall return to these results later when reviewing the studies on the serological properties of the *Escherichia* group. In 1949, Beeuwkes *et al.*, Frisell and Laurell, Holzel *et al.*, Rogers *et al.*, Smith and Taylor *et al.* published the results of investi-

gations in which they established that the incidence of *E.coli var. neapolitanum* is much greater in cases of infantile diarrhoea than in controls. Furthermore, Smith isolated a second sero-type, which he called the β-type to differentiate it from the previous α-type *(E.coli var. neapolitanum)*. Kauffmann and Dupont in 1950 established the identity of the strains isolated in different countries, England (Giles, Smith, Taylor), Holland (Ten Seldam), United States (Ferguson) and Denmark. Of the 38 strains studied, eleven were of the β-type of Smith, which is designated as 55:B5 according to the *Escherichia scheme* of Kauffmann, Knipschildt and Vahlne, while 27 were of the *E.coli var. neapolitanum (α-)* type designated as 111:B4. In the same year, Adam and Aust established that the dyspepsia coli types A IV and A I were identical with the sero-types 111:B4 and 55:B5, respectively. The above-mentioned investigations have led to series of studies conducted in many European countries, in the United States and in Japan.[1] The series published up to 1953 are listed by Braun (1953) in an excellent review on the problem of the pathogenicity of *Escherichia coli* in infants. These studies resulted in the isolation of previously unknown *E.coli* sero-types from cases of infantile diarrhoea. For instance, several investigators (Braun and Resemann 1952, Ørskov 1951, Taylor and Charter 1952), who had no knowledge of each other's work, isolated *Escherichia types* which were later designated as *E.coli* 26:B6 and 86:B7 (Ørskov 1951, 1954). Taylor and Charter (1952) isolated two further *Escherichia* types (E 611 and Canioni) with capsular antigens. When Ørskov examined the *Escherichia* strains isolated from cases of diarrhoea by Biering-Sørensen *et al.* (1947), he found besides strains of the 26:B6 type, also 44:K? strains. Rantasalo and Hallman (1953) found in their series as many diarrhoea cases harbouring the 44:K? type as cases bearing 111:B4 and 55:B5 types together. They regarded as doubtful whether *E.coli* 44:K? is capable of effecting diarrhoea. Of interest are the reports of Ørskov (1951) and Fey (1952) that *E.coli* 55:B5, 26:B6 and 86:B7 types were isolated from cattle with mastitis.

For the sake of convenience, in the following the *Escherichia coli* types 111:B4, 55:B5, 26:B6, 44:K?, 86:B7, E 611 and Canioni will be referred to as *diarrhoeal Escherichia types* and the diar-

[1] Hiroki (1953).

rhoeal cases from which these types have been cultured as *Escherichia diarrhoea cases.*

Whenever a diarrhoeal *Escherichia* type has been isolated in investigations dealing with epidemics, this type has been found in almost all the diarrhoea cases (Bray and Beavan 1948, Giles and Sangster 1948, Beeuwkes *et al.* 1949, Frisell and Laurell 1949, Giles *et al.* 1949, Magnusson *et al.* 1949, 1950, Rogers *et al.* 1949, Taylor *et al.* 1949, Kirby *et al.* 1950, Braun and Henckel 1951, Buttiaux *et al.* 1951, Dupont 1951 *a, b,* Laurell *et al.* 1951, Rogers 1951, Laurell and Lycke 1952, Braun and Resemann 1952, Taylor and Charter 1952, Neter *et al.* 1953 a, Wheeler and Wainerman 1953, Wright *et al.* 1953). In those investigations where all sporadic cases of diarrhoea admitted to the hospital have been included, the frequency of positive cases has been smaller than in the above series and has varied from 0 to 63 per cent (Giles *et al.* 1949, Holzel *et al.* 1949, Smith 1949, Cefalú and Bavastrelli 1950, Smith *et al.* 1950, Cathie and MacFarlane 1951, Grönroos 1951 *a, b,* Hesselberg and Oeding 1951, Neter *et al.* 1951, Schiavini 1951, Williams 1951, Young *et al.* 1951, Adamson 1952, Alexander *et al.* 1952, Cominazzini and Andreoni 1952, Lattes and Colombo 1952, Ross 1952, Shanks and Studzinski 1952, Gyengési and Bodó 1953, Hoster and Krüger 1953, Keller and Marget 1953, Krepler and Zischka 1953, Kröger and Dölle 1953, Kundratitz and Gross 1953, Rantasalo and Hallman 1953, Stoppelman and van der Plaats 1953). The occurrence of diarrhoeal *Escherichia* strains in the control cases in all of these series is of lower order; among 3603 controls under two years of age, *E.coli* 111:B4 was found in 5.7 per cent, among 2436 infants of the same age,*E.coli* 55:B5 was found in 4.1 per cent, and among 1189 controls under two years *E.coli* 26:B6 was found in 0.9 per cent. With only a few exceptions (Payne and Cook 1950, Cathie and MacFarlane 1951, Adamson 1952, Stoppelman and van der Plaats 1953), the investigators have considered that diarrhoeal *Escherichia* sero-types 111:B4, 55:B5, 26:B6 and 86:B7 are associated with infantile diarrhoea.

Diarrhoeal *Escherichia coli* types have not been cultured from human infections other than diarrhoea. In one case showing intestinal and meningeal symptoms reported by Drimmer-Herrnheiser and Olitzki (1951), *Escherichia coli* 111:B4:12 was simultaneously isolated from the faeces and the cerebrospinal fluid. In the exten-

sive series of Kauffmann, Knipschildt and Vahlne where the examined *E.coli* strains were cultured from pathological conditions, no diarrhoeal *Escherichia* strains were isolated. Neter *et al.* (1951) have studied 608 *E.coli* strains cultured from a variety of sources with the exception of patients having infantile diarrhoea without finding the diarrhoeal *Escherichia* types 111:B4 and 55:B5.

THE SEROLOGY OF THE ESCHERICHIA GROUP AND THE SEROLOGICAL AND BACTERIOLOGICAL PROPERTIES OF THE DIARRHOEAL ESCHERICHIA SERO-TYPES

The various attempts made to identify different *Escherichia* strains by serological methods (Burk 1908, Christiansen 1917, Hees 1926, Mikkelsen 1927, Nissle 1929, Ryti 1931, Kyrki 1936, 1944, Sievers 1937, Hayashi 1938) were practically speaking unsuccessful until Kauffmann (1943, 1944) discovered a thermolabile antigen, the L antigen. This L antigen prevented the agglutination of the living and formalin-treated *Escherichia* strains in the homologous O immune serum, which finding provided an explanation for the previous discordant results. The L antigen is destroyed by heating one hour at 60° C. and by alcohol treatment, whereupon the strain is agglutinated by O serum. Many of the O inagglutinable strains were not agglutinated by O sera even after the heat treatment; these strains were designated A forms by Kauffmann. This property was later ascribed to a thermostable A antigen (Knipschildt 1945). A two-hour period at 120°C. was required to destroy its power to prevent agglutination by O serum (Vahlne 1945). Knipschildt established also the existence of a third antigen, the thermolabile B antigen. The latter differed from the L antigen in that its agglutinin-binding capacity was not destroyed by heating. The A, B and L antigens are usually referred to as the K antigens (Kauffmann 1951) and are known as the capsular (A) and envelope or surface (B and L) antigens. The thermolabile flagellar H antigen of *Escherichia* has been shown to be monophasic (Kauffmann 1951).

The K antigens associated with *Escherichia* strains isolated from cases of infantile diarrhoea are mainly of the B type.

The antigenic formulae of the four most frequently reported diarrhoeal *Escherichia* types are 111:B4, 55:B5 (Kauffmann and Dupont 1950), 26:B6 (Ørskov 1951) and 86:B7 (Ørskov 1951). In addition to the non-motile *E.coli* 111:B4 strains (Giles and Sangster 1948, Taylor *et. al.* 1949), strains with H antigens 2 and 12 (Kauffmann and Dupont 1950) and 21 (Le Minor 1953) have been reported. H antigens 6 (Kauffmann and Dupont 1950), 2 (Laurell 1952 *b*), 7 (determined by Ørskov for a strain originally isolated by Krepler and Zischka (Braun 1953)), 11 and 21 (Le Minor 1953) have been established in motile 55:B5 strains. When motility was observed in the 26:B6 sero-type, only the H 11 antigen was demonstrated (Ørskov 1951). The O, B and H antigens of the Canioni type and the O and B antigens of the E 611 type have not been classified. The H2 antigen has been established for the latter type (Charter and Taylor 1952). No reports are to be found on the H antigens of E.coli 86:B7 and 44:K? types.

The importance of determining H antigens in epidemiological studies has been particularly stressed by Wright and co-workers (Wright 1953, Wright *et al.* 1953 and Wright and Villanueva 1953 *a, b*).

The haemolytic power of diarrhoeal *Escherichia* strains has been tested by several workers. The 111:B4 strains isolated by Giles and Sangster (1948) all produced haemolysis on blood agar. In contrast to the findings of Kauffmann and Dupont (1950), Taylor *et al.* (1949) observed that six 111:B4 strains were able to lyse horse red cells, and Laurell *et al.* (1951) that five strains of the same type were able to do the same. The 55:B5 and 26:B6 strains have not been found to produce haemolysins.

Reference should perhaps also be made to the observation of Neter *et al.* (1952 *a, b, c*) that red cells which had been treated with boiled broth cultures of *Escherichia* 111:B4 and 55:B5 and filtrates of the cultures were agglutinated by the homologous sera. Unheated broth cultures of the strains were only slightly effective. This "indirect bacterial agglutination" (Neter *et al.* 1952 *a)* refers to the specific agglutination of red cells produced by antibacterial antibodies acting on the antigen adsorbed onto the red cells.

On the basis of their biochemical reactions, the strains described by Bray belonged to the *E.coli neapolitanum* group according to the classification of Winslow *et al.* (Bray 1945).

TABLE 1

FERMENTATION OF ESCHERICHIA TYPES FROM INFANTILE DIARRHOEA (ACCORDING TO KAUFFMANN, 1951

TABLE 1

FERMENTATION OF ESCHERICHIA TYPES FROM INFANTILE DIARRHOEA (ACCORDING TO KAUFFMANN 1951 AND ØRSKOV 1951)

Fermentation type Sero-type	1 111:B4:2	1 111:B4:—	2 111:B4:12	3 55:B5:6	1 26:B6:—	2 26:B6:11	3 26:B6:—
Adonitol	—	—	—	—	—	—	—
Dulcitol	$+^{2-3}$	$+^{2-4}$	$+^{3-4}$	$+^{1-2}$	$+^{2-3}$	—	—
Sorbitol	$+^{1-3}$	$+^{1-5}$	+	—or×	+	+	+
Sorbose	—	—	—	$+^{1-2}$	+	+	+
Arabinose	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	+
Rhamnose	+	+	$+^{2-9}$	+	+	—	—
Maltose	+	+	$+^{1-2}$	$+^{3-9}$	+	+	+
Salicin	$+^{2-4}$	$+^{2-4}$	$+^{1-2}$	—	×	×	×
Inositol	—	—	—	—	—	—	—
Lactose	+	+	+	+	+	+	+
Sucrose	$+^{1-2}$	$+^{1-2}$	—	+	$+^{2-3}$	$+^{2-3}$	$+^{2-3}$
Mannitol	++	++	++	++	++	++	+—
Glucose	++	++	++	++	++	++	+—
Trehalose	+	+	+	+			
Indole	+	+or—	+	+	+	+	+
H_2S	—	—	—	—	—	—	—
Gelatin	—	—	—	—	—	—	—
Ammonium glucose	+	+	+	+	+	+	+
Ammonium citrate	—	—	—	—	—	—	—
KNO_3	+	+	+	+	+	+	+
Voges-Proskauer	—	—	—	—	—	—	—
Methyl-red	+	+	+	+	+	+	+
Urea	—	—	—	—	—	—	—

Key: + = positive after 1 day; $+^{2-3}$ = positive after 2—3 days; — = negative after 30 days; × = late and irregularly positive or negative; ++= acid and gas

The biochemical reactions of the 111:B4, 55:B5 and 26:B6 types described by Kauffmann and Dupont (1950) and Ørskov (1951) are presented in Table 1. Bray isolated 10 strains of type 111:B4 which differed from the preceding in that they were initially lactose-negative, but on subculture in lactose broth began to ferment lactose. In the series of Laurell *et al.* (1951), there were eight strains of type 111:B4 which liquefied gelatin.

Le Minor (1953) reported several strains of diarrhoeal *Escherichia* types differing in their fermentative reactions from the types listed in Table 1. There were 3 indole-negative 111:B4:2 strains, 11 dulcitol-negative 111:B4:12 and one 26:B6:11 strain which decomposed urea. The single 55:B5:11 strain isolated was adonitol-positive.

STUDIES ON THE PATHOGENICITY OF DIARRHOEAL ESCHERICHIA STRAINS

It has not been found possible to determine by animal experiments the pathogenicity of diarrhoeal *Escherichia* strains as prescribed by the third postulate of Henle-Koch (Braun *et al.* 1953). Their pathogenicity has, however, been demonstrated in tests conducted with human subjects. Neter and Shumway (1950) reported that ingestion of milk infected with *Escherichia* 111:B4 by an infant with multiple congenital defects resulted in diarrhoea. Ferguson and June (1952) administered milk infected with *Escherichia* 111:B4 and 55:B5 to adult volunteers. When the bacteria content of the ingested milk was sufficiently high, all the persons under test developed gastro-intestinal symptoms. By similar feeding tests, Kirby *et al.* (1950) and Braun and Henckel (1952) effected disease of gastro-enteric nature in adults. According to Braun (1953), the ingestion of *Escherichia* 26:B6 gave no response in his subjects, probably owing to the low bacterial dose in his tests.

One of the measures of the pathogenicity of a bacterial strain is its ability to incite antibody production. Antibody studies conducted in cases of infantile *Escherichia* diarrhoea have mainly yielded poor results and the agglutinin titres of sera taken during convalescence have usually been low (Giles and Sangster 1948, Taylor *et al.* 1949, Buttiaux *et al.* 1951, Grönroos 1951 *b*).

Clement *et al.* (1952) established the highest agglutinin titres on the eighth day of disease on the average, the titres ranging from 1:50 to 1:200. Bray (1945) detected antibodies for *E.coli* 111:B4 in only one of ten sera, the titre being 1:320, and Laurell *et al.* (1951) for the same type in 20 out of 54 sera, the titres ranging from 1:40 to 1:100. Braun and Henckel (1951) noted antibodies for 111:B4 in 4 of 34 sera and antibodies for 55:B5 in 7 of 21 sera, the highest titre being 1:2280. Smith *et al.* (1950) examined sera of 98 patients of whom 29 were cases of diarrhoea associated with *E.coli* 55:B5; they found H and O agglutinins in the sera of eight of the latter patients, the highest titre being 1:5280. Ferguson and June (1952) observed that the antibody titres were higher in human subjects after they had ingested milk infected with *Escherichia* 111:B4 and 55:B5, whereas Kirby *et al.* (1950) and Braun and Henckel (1952) were not able to confirm this in their studies.

Neter *et al.* (1953 *b*) established that sera of volunteers who had received *Escherichia* 55:B5 agglutinated red cells treated previously with boiled suspensions of *Escherichia* 55:B5 and that the bacterial haemagglutinin titres were higher in the postfeeding sera.

THE DRUG SUSCEPTIBILITIES OF DIARRHOEAL ESCHERICHIA STRAINS

It is natural that when diarrhoeal *Escherichia* types have been associated with diarrhoeal conditions in infants, the patients have been subjected to specific treatment. Although numerous clinical trials of the effect of various therapeutic agents have been carried out, little attention has been given to their effect *in vitro*.

Gutheil (1951) found that 13 out of 14 isolated *Escherichia* 111:B4 strains were resistant to sulphaguanidine. Twelve strains of the same type tested by the present author (1951 *a)* were sensitive to sulphathiazole. Le Minor (1953) established, however, that of 350 *Escherichia* 111:B4, 55:B5 and 26:B6 strains, 32 111:B4 and 16 55:B5 strains were resistant to sulphonamides and 55 111:B4 strains were found to be moderately sensitive or resistant to the agent.

Studies on the effect of antibiotics *in vitro* have shown that the *Escherichia* 111:B4, 55:B5 and 26:B6 strains are sensitive to

chloramphenicol, chlortetracycline and oxytetracycline (Kauffmann and Dupont 1950, Neter and Shumway 1950, Neter *et al.* 1951, Grönroos 1951 *a*, Gutheil 1951, Ferguson *et al.* 1951, Ørskov 1951, Braun and Henckel 1952, Braun 1953, Le Minor 1953, Smith and Galloway 1953). With the exception of Kauffmann and Dupont, these authors have found many strains resistant to streptomycin in their material. On the other hand, it has been demonstrated by *in vitro* experiments that the development of resistance to streptomycin is accompanied by an increased sensitivity to the action of chloramphenicol, chlortetracycline and oxytetracycline (Kaipainen 1951).

Among 32 investigated *Escherichia coli* 111:B4 strains, Ferguson *et al.* (1951) found 18 salicin-negative strains to be highly resistant to streptomycin, while salicin-positive strains were comparatively sensitive to the antibiotic.

Ferguson *et al.* (1951) established also that neomycin and polymyxin were effective against all the 32 *Escherichia* 111:B4 strains tested. Similar results were obtained by Neter and Shumway (1950) with polymyxin in the case of 9 strains of the same type.

Gorzynski and Neter (1953) determined the *in vitro* susceptibility to streptomycin and neomycin of 29 strains representing *Escherichia* sero-types 111:B4, 55:B5 and 26:B6. All the strains were sensitive to neomycin, whereas seven were resistant to streptomycin. No influence of the streptomycin resistance on the neomycin sensitivity could be observed.

EPIDEMIOLOGICAL AND CLINICAL FEATURES OF ESCHERICHIA DIARRHOEA

Attempts made to determine the way in which infantile *Escherichia* diarrhoea spreads outside hospitals have been unsuccessful. Hospital epidemics, however, have contributed greatly to the elucidation of the mode of contagion in these institutions (Smith *et al.* 1950, Braun and Henckel 1951, Laurell *et al.* 1951, Rogers and Koegler 1951, Laurell 1952 *a*, Schmidt *et al.* 1952). The hospital epidemics have not been explosive by nature. The incubation time of *Escherichia* diarrhoea has varied in

different series: it is mainly limited to 10 days, the shortest times reported being 2—4 days (Rogers *et al.* 1949, Braun and Henckel 1951, Laurell *et al.* 1951, Ocklitz and Schmidt 1952). Diarrhoeal *Escherichia* types have been recovered from the ward already within several hours after a patient infected with a diarrhoeal *Escherichia* type has been admitted. The bacteria are found in the air, dust, clothing and wash basins and on the garments of the nursing staff (Braun and Henckel 1951, Rogers 1951, Schmidt *et al.* 1952, Müller 1953).

Isolation of diarrhoeal *Escherichia* sero-types from the respiratory tract has been reported by several investigators (Taylor *et al.* 1949, Neter and Shumway 1950, Grönroos 1951 *b*, Laurell *et al.* 1951, Braun and Henckel 1952). Laurell *et al.* 1951 have in several cases established diarrhoeal *Escherichia* strains in the respiratory tract before diarrhoea symptoms have become evident. Diarrhoeal *Escherichia* strains have been isolated also from nose swabs taken from the nursing staff (Laurell *et al.* 1951).

Flies have been found to carry diarrhoeal *Escherichia* strains by Bray (1945) and Ocklitz and Schmidt (1952). The spread of *Escherichia* diarrhoea by flies was stressed by the first author.

The seasonal incidence of *Escherichia* diarrhoea has been studied by several investigators, who observed a prevalence in Winter and early Spring (Bray 1945, Giles and Sangster 1948, Giles *et al.* 1949, Kirby *et al.* 1950, Braun and Henckel 1951, Laurell *et al.* 1951, Rogers 1951, Hoster and Krüger 1953). In only a few studies have epidemics been encountered in other seasons (Drimmer-Herrnheiser 1951, Krepler and Zischka 1953). In the series of Rantasalo and Hallman (1953), the cases were evenly spread over the whole year. The occurrence of different diarrhoeal *Escherichia* types has been found to vary in different years (Braun 1953, Smith 1953).

The examination of the age incidence of infantile diarrhoea cases associated with E.coli 111:B4, 55:B5 and 26:B6 reveals that the incidence is higher in the first and second trimesters (Giles *et al.* 1949, Kirby *et al.* 1950, Rogers *et al.* 1949, Taylor *et al.* 1949, Braun and Henckel 1951, Laurell *et al.* 1951, Smith 1949, Modica *et al* 1952, Hoster and Krüger 1953, Rantasalo and Hallman 1953, Ocklitz 1954).

The clinical features of infantile diarrhoea associated with diarrhoeal *Escherichia* types reported and described by various investigators do not differ essentially from those of an ordinary non-specific diarrhoea, including different degrees of severity. The death rates and number of toxic cases reported in papers published during the 1940's have been relatively high, whereas the corresponding data in later series have been much lower in comparison.

As no attention has been paid in the present investigation to pathological-anatomical changes in *Escherichia* diarrhoea, reference is made to the instructive studies of Adam and Froboese (1925), Adam (1952), Biesalski (1952) and Ilgner (1953), which have revealed the strong affinity of the diarrhoeal *Escherichia* types to the epithelium of the small intestine. The resulting changes were of an inflammatory nature.

SUMMARY

The role of bacteria of the *Escherichia* group as causative organisms in infantile diarrhoea has been the subject of considerable discussion during the present century. After the elucidation of the serological properties of the *Escherichia* group, whereupon the group could be divided into clearly defined sero-types, numerous investigators in different countries were able to establish that *Escherichia* coli sero-types 111:B4, 55:B5, 26:B6 and 86:B7 were more frequently present in the faeces of diarrhoeal patients than in those of non-diarrhoeal and healthy children. Most of the investigators have come to the conclusion that these sero-types are closely associated with infantile diarrhoea although these same types have also been isolated from the controls, who have mainly been patients hospitalized for other conditions.

It has not been possible to determine the pathogenicity of the diarrhoeal *Escherichia* types by means of animal experiments, but volunteers who have received sufficiently large doses of 111:B4 and 55:B5 strains have reacted with gastro-intestinal symptoms. The pathogenicity has not been defined by agglutination studies with human sera since bacterial antibodies have been only infrequently established in the sera of the diarrhoeal patients. Bacterial haemagglutinins, however, have been demonstrated in

conducted feeding experiments, positive results being obtained even when ordinary bacteria agglutination has not succeeded.

Most of the studies have, however, been conducted with small series of patients during short periods of time and furthermore these have primarily dealt with epidemics. Only seldom has any greater attention been paid to the serological and bacteriological properties of the isolated *Escherichia* strains.

II. THE PRESENT STUDIES

A. METHODS

THE BACTERIOLOGICAL TECHNIQUE

Collection of specimens and culture methods. — The faecal specimens were collected from the patients on admission and 1—3 times weekly in the mornings during their stay in the hospital. If evacuation had occurred, specimens were taken with swabs from stools in the diapers, and if not, rectal swabbings were made. The swabs were stored inserted in the cork stoppers of 20-ml vials which contained about 4 ml of physiological saline to prevent drying of the specimen. The specimens from both the Children's Clinic and the Epidemic Hospital were cultured within a few hours after they had been taken. For the follow-up study faecal specimens (mostly mixed with urine) were collected and forwarded to the laboratory by health nurses.

The specimens were cultured on the following media:

1. Bromo-cresol purple lactose agar:
   Meat extract 1000 ml, peptone 5 g, agar 20 g, lactose 15 g, bromo-cresol purple 1 ml of 1.6 per cent solution in alcohol.
2. Bromo-cresol purple agar containing 0.1 per cent sodium desoxycholate (Grönroos 1950 *b*) and 0.1 per cent sodium thiosulphate.
   or SS-agar (Difco)
3. Enrichment medium, either the tetrathionate broth of Kauffmann (1951) or selenite F broth of Leifson (1936).

When rectal swabs were forwarded, a small area of a plate 10 cm in diameter was inoculated with the swab and the inoculum spread with a platinum wire loop. When the specimen comprised only faecal matter, one platinum loopful was inoculated onto the plate and spread with a sterilized platinum loop. A selective bromo-cresol purple desoxycholate or an SS-agar plates was cultured first, so that these were more heavily inoculated. The enrichment medium was inoculated with a loopful

or with 1—2 ml of a saline suspension of the specimen. The selective medium (2) was inoculated with one loopful of the inoculated enrichment medium after overnight incubation.

The nose and throat specimens were taken with swabs in the mornings and cultured on blood agar and on bromo-cresol purple agar plates (1).

Identification technique. — The actual occurrence of diarrhoeal Escherichia strains was examined using the the bromo-cresol purple lactose agar plates (brcp-plate), while the selective and enrichment media were employed to recover possible *Salmonella* and *Shigella* strains. The method used to identify Salmonella and Shigella types has been previously described by the author (1953).

The existence of diarrhoeal *Escherichia* colonies on the brcp-plates was studied after overnight incubation at 37°C. Colonies were preliminarily agglutinated on slides in immune sera of various diarrhoeal *Escherichia* types. If the first colony happened to give a positive result, no further colonies were agglutinated. When the result was negative, as many as five colonies were agglutinated unless the colonies were clearly atypical. At first only the occurrence of *E.coli* 111:B4 was examined. The other strains were included later, as these were reported in the literature. *E.coli 111:B4 was hence diagnosed during the whole duration of the investigation, from 1 March 1950 to 2 October 1953, E.coli 55:B5 from 1 Jan. 1951, 26:B6 and 44:K? from 2 May 1951, 86:B7 from 26 September 1952 and E 611 and Canioni from 13 February 1953 onwards.*

From 1 October 1952 eight coliform colonies from each of the primary brcp-plates were subcultured twice and agglutinated on a slide with a mixed serum (see p. 27). When a strain agglutinated on the slide, it was again subcultured and control agglutination tests were performed in tubes using antigens of the strain in question. When the results were positive, the strain was transferred to egg medium (Kauffman 1941) for storage.

The following biochemical reactions of the strains were studied:

Fermentation reactions:

Monosaccharides:
- Pentoses—
  - Arabinose
  - Xylose
  - Rhamnose
- Hexose—
  - Glucose (Durham tube)

Disaccharides:
- Sucrose
- Maltose
- Lactose
- Trehalose

Alcohols:
- Pentahydric—
  - Adonitol
- Hexahydric—
  - Mannitol (Durham tube)
  - Dulcitol
  - Sorbitol

Glucoside:
- Salicin

Non-carbohydrate substance:
- Inositol

The culture media containing carbohydrates or inositol were prepared according to Kauffmann, except that a 1.6 per cent solution of bromocresol purple in alcohol was employed as indicator. The inoculated tubes were incubated at 37°C. and the strains were marked positive when the colour changed and negative when no colour change was observed after an incubation period of 30 days. *All exceptional reactions were retested.*

Indole production was studied using casein broth (Kauffmann 1951) to which Ehrlich's reagent was added after overnight incubation.

The methyl-red and Voges-Proskauer reactions were studied in a glucose broth. In the former case, the change in pH was studied by adding methyl red in alcohol to the culture after overnight incubation. In the latter case, the formation of acetyl-methyl-carbinol was detected by following during one day the colour change effected by addition of 10 per cent sodium hydroxide to a four-day culture (Thjøtta 1946).

Nitrate reduction was tested according to Kauffmann (1951).

Urea decomposition was studied using sloped agar containing urea (Christensen 1946).

Citrate utilisation was determined using Simmon's citrate-containing sloped agar (Thjøtta 1946).

Hydrogen sulphide production and *liquefaction of gelatin* was tested in a gelatin medium containing ferrous sulphate. The instructions of Kauffmann (1951) have been modified of necessity, but only to the extent that peptone from Roskilde Medical Company Ltd. was substituted for that from Parke, Davis and Co. and ferrous chloride was replaced by ferrous sulphate.

The *motility* of the diarrhoeal Escherichia strains was followed in a 0.1 per cent agar medium in a U-tube (Wahlne 1945) or in a Craigie tube (Craigie 1931) at 37°C. The latter tube is much more convenient to use and it was necessary to adopt this tube when large numbers of cultures were being examined since it was difficult to keep the U-tubes in order. When a strain did not exhibit motility during the first day, it was transferred to grow in a broth medium, from which a U-tube or a Craigie tube was repeatedly inoculated. If growth throughout the medium was not detected in the tube after five passages, the strains were reinvestigated after they were kept in Craigie tubes for one week at room temperature (Wright and Villanueva 1953 *b.)*. If no swarming through the medium occurred, the strain was considered non-motile.

The *haemolytic power* of the diarrhoeal *Escherichia* strains was tested according to Widholm (1953). The haemolysis of sheep red cells in one per cent peptone (Alwitt Trading Co. Ltd, London) water was examined after 24 hours' incubation at 37°C.

THE SEROLOGICAL TECHNIQUE

Preparation of immune sera. — The immunisations were carried out using rabbits which were close to one year old. The antigens *(vide infra)* were injected intravenously in volumes of 0.25, 0.50

and 1.0 ml at intervals of five days. Four days after the last immunisation, a blood specimen was taken and if the titre was sufficiently high, the animal was bled on the next day by severing the carotid artery. If the titre was low, the immunisation of the rabbit was continued in the same manner using antigen injections of 1.5—2 ml. After the retraction of the coagulum, the blood was centrifuged, the serum was removed and, after adding merthiolate (Lilly), placed in vials and inactivated for half an hour at 56°C. The immune sera were stored at + 4°C.

O sera were made using overnight broth cultures steamed for 2½ hours, and H sera using overnight formolized broth cultures. For the production of K immune sera, cultures were incubated for 18 hours on thick 0.1 per cent glucose agar and suspended in 5 ml of physiological saline solution. The K antigen suspensions were prepared immediately before each immunisation.

The immune sera were prepared with strains D 433 (111:B4: —) supplied by Prof. G. Olin, State Bacteriological Laboratory, Stockholm, with 1064 (55:B5:6), 26:B6:—, 44:K? and E 990 (86:B7) type strains received from Dr. F. Kauffmann and Dr. F. Ørskov of the International Salmonella and Escherichia Centre and with E 611 and Canioni strains from Dr. J. Taylor, Central Public Health Laboratory, Colindale, London. The H immune sera 1—33 were prepared using strains sent by Dr. F. Ørskov.

The agglutination and absorption techniques. — Slide agglutination tests with OK immune sera were performed only as screening tests on strains from the subcultures. When monovalent sera were used, the dilution in slide agglutination tests was 1:20. In the case of polyvalent sera, the serum taken was such that its final dilution was 1:10.

The O, K and H agglutination tests were all performed in test tubes using volumes of 0.2 ml. The dilution series of the O and H agglutination tests were incubated in a water-bath at 50°C., overnight for the former and two hours for the latter test. The evaluation was performed after the tubes were removed from the water-bath (Kauffmann 1951).

In the K agglutination tests, the tubes were incubated at 37°C. for two hours and were examined after they had stood overnight at room temperature. The titre of the strain under study was compared with that of a control strain in each test.

To confirm that a strain possessed a K antigen, the O inagglutinability of the living or formolized suspension of an agar culture was determined by tube agglutination tests with O immune sera. The reaction was examined after incubating for 2 hours in a water-bath at 37°C. and storing at room temperature overnight. When no O agglutination was observed, the strains was considered to possess a K antigen when a parallel O agglutination test yielded the titre usually obtained with the O immune serum.

The identity of various antigens and agglutinins were determined by cross-absorption tests. These were carried out by following closely the directions of Kauffmann (1951).

Comparison of the results of the slide and tube agglutination tests. — In order to determine the reliability of the slide agglutination tests, 644 bacterial strains from stool cultures were tested for agglutination on slides with polyvalent and monovalent immune sera and in tubes with monovalent immune sera only. 324 of the strains were found to give negative results in the tube tests. In the slide tests, 211 or 65.5±2.6 per cent of these yielded positive results. Of the positive strains, 7 or 2.2±0.8 per cent were negative in the slide tests. It is thus obvious that agglutinations conducted on slides must be confirmed by tube tests. It may be noted, however, that the slide agglutination tests can be relied upon to give a separation of the positive strains for later tube agglutination tests with an almost 100 per cent certainty, particularly as more than one colony is usually tested when agglutination does not occur.

THE IDENTIFICATION OF ANTIBODIES IN SERA OF PATIENTS

In order to detect possible *agglutinins* for diarrhoeal *Escherichia* strains in the serum of a patient, the serum specimens were diluted in twofold dilutions beginning with the dilution 1:10 and agglutination tests were performed as described with the O, H and K antigens of the homologous strain isolated from the patient.

Possible *incomplete antibodies* for diarrhoeal *Escherichia* strains were determined in some cases using Coomb's technique as modified by Wilson and Merrifield (1951). The test tubes which were found negative in the agglutination tests were centrifuged and the supernatant fluid was removed. The bacteria remaining were washed three times with physiological saline solution and tested in antihuman globulin serum. The agglutination tests were carried out in small 0.6×5.5 cm test tubes.

Possible *absorbed antibodies* on red cells of diarrhoeal infants were determined according to Neter *et al.* (1952 *a, b*). The red cells of the infants were washed three times with physiological saline solution and agglutinated in the O immune rabbit serum corresponding to the strain isolated in 0.6×5.5 cm test tubes. The tubes were examined after incubation for 1 hour at 37°C.

After the 1953 publication of Neter, Zalewski and Ferguson appeared, the remaining sera, which in the meantime had been stored in a refrigerator at —20°C., were tested for the presence of *haemagglutinins*

for *E.coli* 111:B4, 55:B5 and 26:B6 using a technique which only slightly differed from that described by these authors. Human O red cells were washed three times with physiological saline and treated with a boiled broth culture of the homologous Escherichia strain isolated from the infant whose serum was to be tested. The modified cells were again washed three times and mixed with equal volumes (0.2 ml) of serial dilutions of the sera. The mixtures were incubated at 37°C. for 1 hour and examined grossly. To check the specificity of the results, the sera were also tested in the same manner for the presence of bacterial haemagglutinins for a heterologous diarrhoeal *Escherichia* strain.

THE TESTING OF THE DRUG SUSCEPTIBILITY

The drug sensitivities of the diarrhoeal Escherichia strains were evaluated by the plate dilution method (Waksman and Reilly 1945) using a peptone-free agar medium with pH 7.2—7.4. The solutions of the therapeutic agents used in these experiments were prepared just prior to the pouring and inoculation of the plates. From 1953, agents from the antibiotic kit donated by Pfizer and Co. were employed. Previously 250 mg capsules of chlortetracycline (Lederle), chloramphenicol (Parke, Davis and Co.) and oxytetracycline (Pfizer and Co.) were used. A 20 per cent sulphathiazole solution (Astra) was employed when studying the action of this drug. The dihydrostreptomycin tests were performed with didromycine (Specia). The neomycin was made available by Lääke Oy., Turku.

The strain under test was inoculated into broth from a 18-hour agar culture. After an incubation period of six hours, the broth was diluted to 1:8000. According to the Standard Opacity Tube series (Burroughs Wellcome and Co.), this dilution contains approximately 100,000 bacteria per ml. From the diluted broth the agar culture media were inoculated with a platinum loop by drawing the serial numbers of the strains on the media. In this way the time required to make the usual markings on the plates was saved. The plates were usually tested after 18 hours' incubation, but in the case of chlortetracycline, the incubation period was 12 hours. The sensitivity of the strain was evaluated by noting the concentration of the therapeutic agent which completely inhibited the growth.

The *Escherichia coli* 2876 strain and the strain *Salmonella paratyphi B* 3142/49 isolated by the author (1950 *a*) are used routinely in the susceptibility tests at our Department. Hence the former was employed as a control strain in the susceptibility tests with streptomycin and the latter strain in tests with chloramphenicol, oxytetracycline, polymyxin B and neomycin. The *Bacillus cereus* strain No. 5 (supplied by Dr. G. Tunevall from The Municipal Bacteriological Central Laboratory, Stockholm)

was employed as a control strain in the susceptibility tests with oxytetracycline and the *Micrococcus pyogenes aureus* Oxford H strain in the tests with sulphathiazole. The growth of these control strains was inhibited by the concentrations of the therapeutic agents given in the following tabulation.

Strain	Therapeutic agent	Inhibition concentration range
Escherichia coli 2876	Streptomycin	1.5—0.8 microg/ml
Salmonella paratyphi B 3142/49	Chloramphenicol	5—2.0 „
Bacillus cereus No. 5	Chlortetracycline	0.1—0.05 „
Salmonella paratyphi B 3142/49	Oxytetracycline	2.0—1.0 „
„	Polymyxin B	12.5—6.2 „
„	Neomycin	5.0—2.0 „
Micrococcus pyogenes aureus Oxford H	Sulphathiazole	5—1.2 mg/100 ml

B. THE PRESENT SERIES

The material of the present investigation was collected during the period 1 March 1950 to 2 October 1953. In all 12045 faecal specimens and 6527 throat and nasal swabs were examined for the presence of diarrhoeal *Escherichia* types. The possible presence of *Shigellae* and *Salmonellae* in the faecal specimens was also investigated. In addition, 1133 *E.coli* strains cultured from pus and urine specimens were examined to determine whether they belonged to the diarrhoeal *Escherichia* types.

Special attention has been paid to a group of infants which included patients under two years treated during the above period at the Children's Clinic of the Turku University and at the Epidemic Hospital of Turku and healthy children under one year selected at random from among those who visited three Child Welfare Centres of the City of Turku during 1953. These infants comprised a homogenous group in that the taking and delivery of specimens was conducted in a uniform manner and in that other information relating to them was readily available. The age distribution in this group in different years is shown in Table 2

TABLE 2

AGE DISTRIBUTION AMONG DIARRHOEAL PATIENTS AND OTHER SUBJECTS OF THE PRESENT SERIES IN DIFFERENT YEARS

Period	Diarrhoeal patients									Non-diarrhoeal patients, healthy children and members of the hospital staffs								
	Age groups									Age groups								
	Under 24 months						Over 24 months			Under 24 months						Over 24 months		
	< 1	1—2	3—5	6—12	13—24	Total	25—60	> 60	Total	< 1	1—2	3—5	6—12	13—24	Total	25—60	> 60	Total
2.2-31.12. 1950	33	68	58	53	8	220	3	—	3	43	38	20	49	7	157	7	40	47
1951	15	57	38	80	20	210	23	2	25	122	121	69	139	43	494	46	148	194
1952	16	50	59	74	54	253	22	6	28	184	122	90	163	40	599	60	172	232
1.1.-2.10. 1953	20	49	41	56	16	182	20	8	28	148	135	144	312	105	844	67	171	238
Total	84	224	196	263	98	**865**	68	16	84	497	416	323	663	195	**2094**	180	531	711

The total number of examined patients and healthy children from the above hospitals and Centres was 3309. These fell into groups as shown in Table 3.

TABLE 3

DISTRIBUTION OF THE SUBJECTS

Group	Diarrhoeal patients			Non-diarrhoeal patients and healthy children			Total
	Under 24 months	Over 24 months	Total	Under 24 months	Over 24 months	Total	
Treated at the Children's Clinic	604	48	652	1713	230	1943	2595
Treated at the Epidemic Hospital	258	36	294	113	36	149	443
Infants from Child Welfare Centres	3		3	268		268	271
Total	**865**	84	949	**2094**	266	2360	**3309**

The data relating to the 865 diarrhoeal children under two years and to the group of 2094 non-diarrhoeal and healthy children of the same age, which was considered as a control group, have been subjected to a detailed analysis. The age distribution in these groups is shown in Table 4. The faecal specimens taken from the

TABLE 4

DIARRHOEAL PATIENTS AND NON-DIARRHOEAL PATIENTS AND HEALTHY CHILDREN UNDER TWO YEARS GROUPED ACCORDING TO AGE

Age in months	Diarrhoeal patients		Non-diarrhoeal patients and healthy children	
	Number	%	Number	%
< 1	84	9.7±1.0	497	23.7±0.9
1—2	224	25.9±1.5	416	19.9±0.9
3—5	196	22.7±1.4	323	15.4±0.8
6—12	263	30.4±1.6	663	31.7±1.0
13—24	98	11.3±1.1	195	9.3±0.6
Total	865	100.0	2094	100.0

first group numbered 4119, those from the second group 5332. The symptoms of patients in the diarrhoea group varied through all degrees of severity.

From the patients and healthy children over 24 months of age 835 faecal specimens were taken.

A further 739 faecal specimens were collected from 445 members and 1287 nose and throat specimens from 276 members of the staffs of the hospitals mentioned above. In all, 11025 faecal specimens from these 3754 patients, healthy children and members of the staffs were examined. The remaining 1020 faecal specimens were from other hospitals and welfare centres.

The serological and bacteriological properties of 907 diarrhoeal *Escherichia* strains isolated from 448 faecal cultures have been analysed.

One hundred and seventy-two sera have been examined for the presence of antibodies for diarrhoeal *Escherichia* strains. The sera were from 155 patients, of whom 98 were positive for diarrhoeal *Escherichia* types, 69 of them established cases of diarrhoea.

One hundred and sixty-five sera were tested for diarrhoeal *Escherichia* haemagglutinins; of these sera 36 were from patients who suffered from *Escherichia* diarrhoea.

C. RESULTS

THE OCCURRENCE OF DIARRHOEAL ESCHERICHIA TYPES IN THE PRESENT SERIES

The number of specimens positive for diarrhoeal *Escherichia* types among the 12045 faecal specimens was 479; of these 350 were specimens from diarrhoea cases. The type distribution is shown in Table 5. The proportion of the faecal specimens positive for diarrhoeal *Escherichia* strains which were from established diarrhoeal patients was 73.2±2.1 per cent.

None of the faecal specimens examined were found positive for *Shigellae*, but *Salmonella paratyphi* B and *Salmonella typhi murium* were isolated in 21 or 2.4±0.5 per cent of the diarrhoeal patients. These latter were all children under two years. It may be mentioned that from the patients infected with *Salmonella*

TABLE 5

TOTAL NUMBER OF SPECIMENS POSITIVE FOR VARIOUS DIARRHOEAL ESCHERICHIA TYPES IN 12045 FAECAL SPECIMENS AND THE NUMBER OF POSITIVE SPECIMENS FROM ESTABLISHED CASES OF DIARRHOEA

Diarrhoeal Escherichia type	Number of positive specimens	Number of positive specimens from established diarrhoea cases	Percentage of positive specimens from established diarrhoea cases
111:B4	180	157	87.2±2.5
55:B5	106	46	43.5±4.7
26:B6	144	122	84.8±3.0
44:K?	48	24	50.0±7.2
E 611	1	1	
Total	479	350	73.2±2.1

strains, *E.coli* 26:B6 was isolated in two cases and sero-types 55:B5 and 44:K? each in one case. In the evaluation of the part played by diarrhoeal *Escherichia* types in infantile diarrhoea, the cases found positive for *Salmonellae* have been disregarded. Consequently the number of diarrhoeal cases under two years old in the present series reduces to 844.

One hundred and seventy-two of the 844 diarrhoeal infants were found positive for diarrhoeal *Escherichia* types. Of these 172 *Escherichia* diarrhoea cases, 145 were treated at the Children's Clinic, 24 at the Epidemic Hospital and the remaining three were infants from Child Welfare Centres. Ninety-two of the 2094 infants in the non-diarrhoeal group were infected with diarrhoeal *Escherichia* strains. Of these 92 cases, 87 were treated at the Children's Clinic and only one at the Epidemic Hospital, while four were infants from Child Welfare Centres. Thus 24.0±1.8 per cent of the diarrhoea patients treated at the Children's Clinic were *Escherichia* diarrhoea cases. This percentage is significantly higher than the 9.3±1.8 per cent obtained for the patients at the Epidemic Hospital. The percentages for the controls treated at respective hospitals are 5.1±0.5 and 0.9±0.8.

The number of diarrhoeal patients positive for *E.coli* 111:B4 was significantly higher in the diarrhoea group (9.5±1.0 %) than in the control group (1.0±0.2 %). Similarly *E.coli* 26:B6

was more frequently found among the diarrhoeal cases (8.7±1.2 %) than among the controls (1.0±0.2 %). Although the number of cases found positive for sero-type 44:K? was smaller than those positive for the above types, this type was also relatively more frequent in the diarrhoea group (3.5±0.8 %) than in the control group (1.0±0.2 %). With respect to the cases positive for strain 55:B5, however, the two groups did not show any significant difference (3.8±0.8 and 2.1±0.3 %) (Table 6).

TABLE 6

THE PERCENTAGE OF CASES FOUND POSITIVE FOR ESCHERICHIA TYPES 111:B4, 55:B5, 26:B6 and 44:K? IN THE DIARRHOEA GROUP AND IN THE GROUP COMPRISING NON-DIARRHOEAL AND HEALTHY CHILDREN

All subjects were under two years of age

Diarrhoeal Escherichia type	Diarrhoeal cases			Non-diarrhoeal patients and healthy children			Difference between percentages
	Total no. of cases	Diarrhoeal Escherichia positive		Total no. of cases	Diarrhoeal Escherichia positive		
		Number	%		Number	%	
111:B4	844	80	9.5±1.0	2094	20	1.0±0.2	8.5±1.0
55:B5	624	24	3.8±0.8	1937	40	2.1±0.3	1.7±0.9
26:B6	564	49	8.7±1.2	1799	18	1.0±0.2	7.7±1.2
44:K?	564	20	3.5±0.8	1799	18	1.0±0.2	2.5±0.8
One of the above types	564	107	19.0±1.7	1799	82	4.6±0.5	14.4±1.8

Two *Escherichia* types were found simultaneously in six cases, types 26:B6 and 55:B5 in two and 55:B5 and 44:K? in four cases; one of the former and one of the latter were diarrhoea cases. These six cases are included in both groups in the type distribution.

No *E.coli* 86:B7 strains were isolated from the patients examined after September 1952. In the cases examined after 13 February 1953, E 611 was found in only one diarrhoea case, whereas the Canioni type was not diagnosed during this period.

In order to obtain some idea of the occurrence of diarrhoeal *Escherichia* types in the whole series of children under two years, the number of cases positive for diarrhoeal *Escherichia* types in the diarrhoea group and in the control group have been compared

with the total number of diarrhoeal cases in each group. In the diarrhoea group, 20.2±1.4 per cent were positive for diarrhoeal *Escherichia* types and in the control group, 4.4±0.5 per cent. When the frequency of diarrhoeal *Escherichia* types is calculated for that part of the series where the identification of all four types was possible, it is found that these types were present in 19.0±1.7 per cent of the diarrhoeal cases and in 4.6±0.5 per cent of the control cases (Table 6).

No diarrhoeal *Escherichia* strains were isolated from the 84 diarrhoea patients who were over two years old. In the group of 266 patients over two years who suffered from other diseases than diarrhoea, types 111:B4 and 55:B5 were each isolated once.

In the specimens taken from infants at the Child Welfare Centres, *E.coli* 26:B6 was found once, 55:B5 twice and 44:K? four times. The first three infants developed diarrhoea after the specimens were taken.

TABLE 7

CLASSIFICATION OF CASES ACCORDING TO WHETHER THEY WERE FOUND POSITIVE FOR DIARRHOEAL ESCHERICHIA TYPES ON OR AFTER ADMISSION TO THE HOSPITALS

Diarrhoeal Escherichia strain	Escherichia diarrhoea cases			Non-diarrhoeal cases		
	Total no. of cases	Found positive		Total no. of cases	Found positive	
		On admission	After admission		On admission	After admission
111:B4	80	12	68	20	1	19
55:B5	22	8	14	40		40
26:B6	48	27	21	18	1	17
44:K?	20	7	13	14	1	13
Total*	168	54	114	88	3	85
%	100.0	32.2±3.6	67.8±3.6	100.0	3.4±1.9	96.6±1.9

* Cases from whom two different types were isolated are counted only once.

From the 739 faecal specimens taken from the 445 members of the hospital staffs, *E.coli* 111:B4 was cultured once, type 55:B5 three times and type 44:K? once. The specimens were from different persons.

When the hospitalized patients positive for diarrhoeal *Escherichia* types are classified according to whether they were found positive on or after admission to the hospital (Table 7), it is found that the numbers of cases positive on admission is substantially larger among the diarrhoeal cases (32.2±3.6 %) than among the non-diarrhoeal patients in whom diarrhoeal *Escherichia* types were found (3.4±1.9 %).

Despite the fact that the occurrence of *E.coli* in cultures made from the 6527 nose and throat specimens was not particularly infrequent (4.5 %), diarrhoeal *Escherichia* types were established in only 10 diarrhoea cases; 111:B4 was found in eight cases and 55:B5 and 44:K? in one case each. No diarrhoeal *Escherichia* types were diagnosed in the 1287 nose and throat specimens from the 276 members of the hospital staffs although the specimens were intentionally taken when there were patients harbouring diarrhoeal *Escherichia* types in the wards.

An indication of the specific nature of the diarrhoeal *Escherichia* types is their absence in urinary and gall bladder infections and suppurative conditions. All the *Escherichia coli* strains, 1133 in number, cultured from 877 cases of urinary infections, from 83 cases of gall bladder infections and from 173 cases with suppurative conditions were other than diarrhoeal *Escherichia* types.

THE BACTERIOLOGICAL AND SEROLOGICAL PROPERTIES OF THE ISOLATED STRAINS

The sero-types of the diarrhoeal *Escherichia* strains isolated from 907 colonies from 448 cultures are given in Table 8. A major part of the strains, 86.6±1.1 %, were of the *Escherichia* types 111:B4:—, 111:B4:12, 55:B5:4, 26:B6:— and 26:B6:11. The 55:B5 sub-type with H 4 antigen has not been previously reported. Neither have the 44:K? sub-types been described in detail.

TABLE 8

THE NUMBER OF ISOLATED DIARRHOEAL ESCHERICHIA STRAINS COLUMNS 3 AND 4 GIVE THE NUMBER OF STOOL CULTURES AND PATIENTS FROM WHICH THE STRAINS WERE ISOLATED

Diarrhoeal Escherichia type	Number of strains		Number of stool cultures		Number of patients	
111:B4:—	121		121		64	
111:B4:2	5		5		1	
111:B4:12	57		40		28	
111:B4:?	4	187	3	169	3	96
55:B5:—	14		6		5	
55:B5:4	414		84		53	
55:B5:6	11		7		5	
55:B5:7	18		4		2	
55:B5:11	1		1		1	
55:B5:?	14	472	4	106	7	73
26:B6:—	144		89		46	
26:B6:11	49		31		19	
26:B6:?	6	199	4	124	5	70
44:K?:—	19		19		17	
44:K?:18	14		14		8	
44:K?:?	15	48	15	48	11	36
E 611 (H2)	1	1	1	1	1	1
Total		907		448		276

To confirm the diagnosis of the 55:B5:4 type, the O, OB and H immune sera have been prepared using two of the isolated strains 1789/53 and 2296/53. O and B cross-absorption tests have been performed with the *E.coli* 55:B5:6 type strain 1064 and the two isolated strains and their immune sera. H absorption tests have been made using the strain 5:L4:4 (U 1/41) as the H 4 strain. Tables 9 and 10 show that sera absorbed with suspensions of living cultures and of cultures steamed at 100°C. for 2½ hours give similar titres when titrated with these strains. In cross-absorption tests, the strains were able to exhaust quantitatively all aggiutinins from each other's immune sera.

TABLE 9

ABSORPTION AND AGGLUTINATION TESTS WITH O AND H IMMUNE SERA OF ESCHERICHIA COLI 55:B5:6 TYPE STRAIN 1064 AND STRAINS NOS. 1789/53 AND 2296/53

Escherichia coli 6:K 2a,2c: 1 (Bi 7458/41) has been employed as negative control strain

Immune sera	Absorbed with living or steamed* culture	Titrated with strain							
		1064		1789		2296		Bi 7458/41	
		living	steamed	living	steamed	living	steamed	living	steamed
O 1064		40	2560	40	2560	80	5120	0	0
	1064	0	0	0	0	0	0	0	0
	1789	0	0	0	0	0	0	0	0
	2296	0	0	0	0	0	0	0	0
	Bi 7453/41	40	5120	40	1280	40	1280	0	0
O 1789		20	2560	20	2560	40	2560	0	0
	1064	0	0	0	0	0	0	0	0
	1789	0	0	0	0	0	0	0	0
	2296	0	0	0	0	0	0	0	0
	Bi 7458/41	0	1280	0	1280	0	2560	0	0
O 2296		20	5120	0	2560	20	2560	0	0
	1064	0	0	0	0	0	0	0	0
	1789	0	0	0	0	0	0	0	0
	2296	0	0	0	0	0	0	0	0
	Bi 7458/41	0	2560	0	2560	0	1280	0	0
OB 1064		640	10240	640	5120	640	10240	0	0
	1064	0	0	0	0	0	0	0	0
	1789	0	0	0	0	0	0	0	0
	2296	0	0	0	0	0	0	0	0
	Bi 7458/41	640	5120	640	5120	640	5120	0	0
OB 1789		640	20480	640	20480	640	20480	0	0
	1064	0	0	0	0	0	0	0	0
	1789	0	0	0	0	0	0	0	0
	2296	0	0	0	0	0	0	0	0
	Bi 7458/41	640	10240	320	5120	320	5120	0	0
OB 2296		640	20480	640	20480	640	20480	0	0
	1064	0	0	0	0	0	0	0	0
	1789	0	0	0	0	0	0	0	0
	2296	0	0	0	0	0	0	0	0
	Bi 7458/41	320	10240	320	10240	320	10240	0	0

* kept 2 ½ hours at 100° C.

TABLE 10

ABSORPTION AND AGGLUTINATION TESTS WITH H IMMUNE SERA OF ESCHERICHIA COLI 5:L4:4 TYPE STRAIN U 1/41 AND STRAINS NOS. 1789/53 AND 2296/53

Escherichia coli 6:K2a,2c:1 (Bi 7458/41) has been employed as negative control strain

H immune sera	Absorbed with living culture	Titrated with living strain			
		U 1/41	1789	2296	Bi 7458/41
U 1/41		6400	3200	3200	0
	U 1/41	0	0	0	0
	1789	0	0	0	0
	2296	0	0	0	0
	Bi 7458/41	3200	3200	3200	0
1789		6400	6400	6400	0
	U 1/41	0	0	0	0
	1789	0	0	0	0
	2296	0	0	0	0
	Bi 7458/41	3200	1600	3200	0
2296		6400	6400	6400	0
	U 1/41	0	0	0	0
	1789	0	0	0	0
	2296	0	0	0	0
	Bi 7458/41	1600	1600	1600	0

The biological reactions of the strains with different antigenic formulae are presented in Table 11. All of the isolated strains fermented arabinose, glucose, maltose, trehalose, mannitol and sorbitol. Most of the strains fermented xylose, and the majority were unable to ferment adonitol and inositol. Non-xylose-fermenting strains occured only among the 44:K? strains; eighteen 44:K?—, eight 44:K?:18 and four 44:K?:? strains failed to attack this carbohydrate. *Adonitol was attacked by one Escherichia 26:B6:— strain, by four 55:B5:? strains and by two 44:K?:18 strains. Inositol was fermented also by the adonitol-positive 26:B6:— strain and by one of the two adonitol-positive 44:K?:18 strains.* Eighteen *E.coli* 55:B5:7 and fourteen 55:B5:— strains attacked adonitol irregularly; both positive and negative reactions were established for these strains. *One E.coli 111:B4:— strain was*

found which split inositol, but was sucrose-negative, and hence differed in its fermentation reactions from the other E.coli 111:B4:— strains. The isolated strains fermented rhamnose, sorbose, sucrose, dulcitol and salicin variably and it was not found possible to differentiate between the diarrhoeal *Escherichia* strains on this basis. All strains produced gas from glucose and mannitol, and were indole-positive, but none was able to liquefy gelatin. The strains did not utilise citrate nor decompose urea, and with the exception of one 55:B5:4 strain, gave a negative Voges-Proskauer test. All strains reduced potassium nitrate and gave a positive methyl-red reaction. With the exception of one 26:B6:11 strain, the strains were H_2S-negative. Disregarding the few exceptional reactions shown by some of the strains, the fermentation reactions of the *E.coli* 111:B4:—, 111:B4:2 and 26:B6:11 strains correspond to those reported in the literature. The fermentation reactions of the *Escherichia* type 55:B5:6 deviate from those described earlier in that it splits sorbose irregularly. Previously described strains of this type have been sorbose-positive. The *Escherichia* 55:B5:6 strains also differed from the others in being unable to ferment sucrose and sorbose, and split rhamnose late or failed to attack it.

None of the investigated *E.coli* 111:B4 and 55:B5 strains were found to be haemolytic. One hundred and forty-three of the 144 *E.coli* 26:B6:— strains and 14 *E.coli* 44:K?:? strains lysed sheep red cells within 24 hours. Haemolytic 26:B6:— strains have not previously been reported.

Strains of the same OK group but with different H antigens were only seldom encountered in the same patient. From two cases in whom *E.coli* 111:B4:— was found, also a strain with an H 12 antigen was isolated; in a further two cases, the H antigen of the motile strain could not be determined. From the cases positive for *E.coli* 55:B5:7, a strain with H 4 antigen was also identified in one case, a non-motile strain was isolated in two cases and the H antigen of the other strain could not be identified in one case.

With only a few exceptions, the *Escherichia* strains isolated from the diarrhoea cases were of the same fermentation type in each case. Among the cases positive for *E.coli* 111:B4:—, there was one in which a sucrose-positive but inositol-negative strain

TABLE 1

BIOCHEMICAL BEHAVIOUR OF ISOLATED ESCHERICHIA COLI S

Sero-type	111:B4:—				111:B4:2	111:B4:12			111:B4:?			55:B5:4		55:B5:6	55:B5:7	55:B5:11
Number of strains	1	117	2	1	5	33	22	2	2	1	1	413	1	11	18	1
Arabinose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Rhamnose	+	+	+	+	+	+1-13	+1-35	−	+2	+	−	+1-5	+	×	×	+
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sorbose		−	+1-11		−	×	×	−	×		−	×	+	−	+	−
Sucrose	+	+1-5	+	−	+	−	−	−	+2	−	+5	+1-2	+	−	+	+
Maltose	+	+1-2	+	+	+	+1-4	+1-2	+1-9	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trehalose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adonitol	−	−	−	−	−	−	−	−	−	−	+14	−	−	−	×	−
Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Dulcitol	+	+1-14	+1-7	+	+5	+	+3-8	+4	+1-3	+4	+7	+	+	+2	+1-2	+
Sorbitol	+	+1-14	+1-8	+4	+12-14	+1-7	+1-2	+2	+1-3	+	+3	+1-10	+	+	+	+
Salicin	−	+1-14	+1-8	+6	+ 2-12	+1-11	−	−	−	+4	+	×	+	−	×	−
Inositol	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−
Gas production	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Indole	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H_2S	−	−	−		−	−	−	−	−	−	−	−	−	−	−	−
Gelatin	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Ammonium citrate	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
KNO_3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Voges-Proskauer	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−
Methyl-red	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urea	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Haemolysis		−	−		−	−	−	−	−		−	−	−	−	−	−

Key: + = positive after 1 day; +1-5 = positive after 1—5 days;
− = negative after 30 days;
× = late and irregularly positive or negative.

ABLE 11

ICHIA COLI SERO-TYPES 111:B4, 55:B5, 26:B6 and 44:K?

55:B5:11	55:B5:—	55:B5:?			26:B6:—			26:B6:11			26:B6:?			44:K?:—		44:K?:18					44:K?:?		
1	14	9	1	4	1	142	1	47	1	1	4	1	1	18	1	8	3	1	1	1	4	10	1
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+2	+	+	+	−	+	+
−	×	×	+	+	−	+1-8	+	−	−	+	−	+	−	+	+	+	+	+	+	+	+	+	+6
−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
−	+1-2	−	+	×	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	−	−
−	+2-10	−	+2	−	+	+1-2	+	+	+	+	+	+	+2	−	+	−	−	+3	+3	+3	−	−	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
−	×	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
−	+1-2	+2	+	−	−	+1-8	+2	−	+13	−	−	+	+2	+2	+	+2-4	+2	+5	+5	+5	+12	+2-7	−
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+3	×	−	+5	+2	+	×	+	+1-11	+2	+	+2-7	+	+2	×	−	−	×	+	+	+	−	−	+
−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	!	−

was accompanied by a sucrose-negative but inositol-positive strain, and also from whom both salicin-positive and salicin-negative strains were isolated. The strains from 10 of the 30 diarrhoea cases from which *E.coli* 111:B4:12 was isolated fermented salicin, while the strains from 12 cases were salicin-negative. In four of the cases, both forms were present. One of the four E.coli 55:B5 strains isolated from one patient was Voges-Proskauer-positive. An *E.coli* 26:B6:11 strain fermenting rhamnose and producing hydrogen sulphide was found in one patient whose other isolated strains were both rhamnose- and H_2S-negative. A case was also found in which one isolated strain split dulcitol, while the other strain was dulcitol-negative. The xylose-positive 44:K?:18 strains, except one of the three sucrose-negative strains, were isolated from the same patient. The exceptional sucrose-negative strain was isolated from a patient from whom also a xylose-negative strain was cultured.

ANTIBODIES FOR DIARRHOEAL ESCHERICHIA STRAINS IN SERA OF PATIENTS

Agglutination tests were carried out with 115 sera from 98 patients positive for diarrhoeal Escherichia strains (Table 12).

TABLE 12

SERA FROM CASES POSITIVE FOR DIARRHOEAL ESCHERICHIA TYPES TESTED FOR O, K AND H ANTIBODIES

Diarrhoeal Escherichia type	Number of sera			Number of patients
	O	K	H	
111:B4	36	37	10	29
55:B5	28	24	16	25
26:B6	43	42	18	36
44:K?	7	8	3	7
E 611	1	1	1	1
Total	115	112	48	98

Sixty-nine of the patients were *Escherichia* diarrhoea cases. The periods when the 85 sera taken from the last-mentioned cases are as follows:

Probable week of disease	I	II	III	IV	>IV
Number of specimens	3 (3)	44 (20)	27 (3)	6 (4)	5 (3)

The figures in brackets give the number of sera which were also tested for bacterial haemagglutinins. O agglutinins were examined in 115, K agglutinins in 112 and H agglutinins in 48 of the serum specimens.

The results obtained in the agglutination tests are summarized in Table 13. In the majority of the specimens, the antibody titres

TABLE 13

THE HOMOLOGOUS AGGLUTININ TITRES OF SERA FROM PATIENTS POSITIVE FOR DIARRHOEAL ESCHERICHIA STRAINS

Agglutinins	Number of sera titrated	Serum dilution					
		1/<10	1/10	1/20	1/40	1/80	1/160
O	115	108	1	1	2	1	2
K	112	102	6	2	1		1
H	48	45	1	1			1

were lower than 1:10. No relation was noted between the few titres rising to 1:10 and higher and the severity of the disease. Agglutinins were found for 111:B4 in six cases, for 55:B5 in two cases and for 26:B6 in seven cases. The H antigens with titres of 1:10 and higher belonged to 111:B4 in one case and to 26:B6 in two cases.

O, B and H agglutinins were found simultaneously in only one serum. The respective titres were 1:10, 1:20 and 1:160. This serum was taken from a diarrhoeal infant positive for 26:B6:11. The serum from one non-diarrhoeal infant was found to contain O111 agglutinin with a titre of 1:80 and B4 agglutinin with a titre of 1:10, whereas the H agglutinin titre was 1:<10.

The sera of a further 57 infants which were negative for diarrhoeal *Escherichia* strains were also examined for the presence of *E.coli* O111, O55 and O26 agglutinins and B4, B5 and B6 agglutinins, but with negative results. Twenty-four of these patients suffered from diarrhoea.

The agglutination of red cells of 25 diarrhoeal infants positive for diarrhoeal *Escherichia* strains in O and OB immune sera homo-

logous to the strain isolated from each case was also investigated, but the results were negative.

Forty sera from *Escherichia* diarrhoea cases were tested to detect incomplete antibodies using the technique described on page 28. No positive results were obtained.

Fifty sera from 44 infants positive for diarrhoeal *Escherichia* strains, 36 of these diarrhoea cases, were tested for the presence of diarrhoeal *Escherichia* haemagglutinins (Table 14). Both the

TABLE 14

ESCHERICHIA 111:B4, 55:B5 AND 26:B6 HAEMAGGLUTININ TITRES OF SERA FROM PATIENTS POSITIVE (I) AND FROM PATIENTS NEGATIVE (II) FOR DIARRHOEAL ESCHERICHIA STRAINS

Serum dilution	I	II		
	Homologous strain	O111	O55	O26
1/<10	11	40	53	38
1/10	6	6	9	5
1/20	6	18	23	18
1/40	2	19	12	16
1/80	7	16	15	23
1/160	7	10	3	14
1/320	1	4	1	1
1/640	8	2		
1/2560	1			
1/5120	1			
Number of sera	50	115	116	115

homologous strain and a heterologous control strain were employed in these tests. The titres established with the homologous diarrhoeal *Escherichia* strains were all higher than those obtained with the heterologous strains. The titres for the heterologous strains did not exceed 1:80. One hundred and fifteen sera from 110 infants negative for diarrhoeal *Escherichia* strains, of whom 33 were diarrhoeal cases, were also tested for presence of *Escherichia* 111:B4, 55:B5 and 26:B6 haemagglutinins. No significant differences were noted between the titres of the sera from the diarrhoeal and the non-diarrhoeal infants. The titres of sera from infants positive for diarrhoeal *Escherichia* strains were in only two cases clearly higher than in the sera from infants negative for diarrhoeal *Escherichia* strains (Table 14).

THE DRUG SUSCEPTIBILITIES OF THE ISOLATED DIARRHOEAL ESCHERICHIA STRAINS

The study of the drug susceptibilities of the diarrhoea *Escherichia* types *in vitro* revealed (Table 15) that 63.8±2.3 per cent of the strains examined were resistant to streptomycin and

TABLE 15

THE SUSCEPTIBILITY OF ESCHERICHIA COLI 111:B4, 55:B5, 26:B6 AND 44:K? STRAINS TO SEVEN THERAPEUTIC AGENTS

Type of strain	No. of strains	No. of patients	Inhibition concentrations for sulphathiazole in mg per cent, for others in microg/ml								Therapeutic agent
			∨ 105	∨ 104	∨ 20	20	10	5	2	0.5	
111:B4	176	99	100	37		1	2		29	7	Streptomycin
55:B5	101	67	89				1		5	6	
26:B6	128	69	56	2		4	1	8	45	12	
44:K?	48	36	5						29	14	
111:B4	177	99					51	89	37		Chloramphenicol
55:B5	103	68						85	18		
26:B6	128	70					3	92	33		
44:K?	48	36						25	23		
111:B4	176	99						74	102		Chlortetracycline
55:B5	104	68						13	91		
26:B6	128	70						66	62		
44:K?	48	36							48		
111:B4	165	99					1	44	120		Oxytetracycline
55:B5	104	68							104		
26:B6	128	70						3	125		
44:K?	48	36			1				47		
111:B4	24	23				2	8	13	1		Polymyxin B
55:B5	98	63				16	10	40	32		
26:B6	44	30			1	31	6	2	4		
44:K?	48	36			1		25	22			
111:B4	57	57							50	7	Neomycin
55:B5	69	50						13	51	5	
26:B6	64	54						1	49	14	
44:K?	48	36						5	40	3	
111:B4	177	99			7	1		96	73		Sulphathiazole
55:B5	104	68			78		1	14	10	1	
26:B6	127	69			6		1	43	77		
44:K?	48	36			1			8	30	9	

that 33.8±2.9 per cent of the strains were not inhibited in a culture medium containing 20 mg of sulphathiazole per 100 ml. Growth arrest was effected with over 99 per cent of the strains by 2—10 microg/ml of chloramphenicol, chlortetracycline and oxytetracycline. The corresponding inhibition concentration range in the determinations conducted with polymyxin B was 2—20 microg/ml. With 92.0±1.8 per cent of the strains tested with neomycin, growth was arrested by 2 microg/ml or less. None of the strains grew on agar containing 5 microg/ml of neomycin.

TABLE 16

THE SUSCEPTIBILITY TO STREPTOMYCIN OF ESCHERICHIA STRAINS OF TYPES 111:B4, 55:B5 AND 26:B6 THE STRAINS ARE GROUPED ACCORDING TO THEIR ANTIGENIC STRUCTURES INCLUDING H ANTIGENS

Diarrhoeal Escherichia type	No. of strains	No. of patients	Inhibition concentrations in microg/ml						
			>10^5	>10^3	20	10	5	2	0.5
111:B4:—	121	65	85	33				3	
111:B4:12	40	30	12	1	1	1		18	7
55:B5:4	82	51	82						
55:B5:—									
55:B5:6									
55:B5:7	14	8	4			1		3	6
55:B5:11									
26:B6:—	94	32	48	2	3	1	6	24	10
26:B6:11	29	9	8				1	18	2

An examination of the diarrhoeal *Escherichia* strains (Table 16) showing resistance to streptomycin revealed that most of the resistant strains belonged to the 111:B4:—, 55:B5:4 and 26:B6:— types. These particular *Escherichia* types were the ones that were found to spread within the hospital (Table 20), a fact that should be noted.

The variation of the drug susceptibilities of the diarrhoeal *Escherichia* strains during treatment could be followed in 46 cases. Only the susceptibility to streptomycin was observed to change. Resistance developed during treatment in 12 cases, in 30 cases the strain was resistant when first isolated and in four cases the susceptibility to streptomycin did not undergo any change during

the treatment. In the first 12 cases, streptomycin was administered in oral doses of 100—200 mg three times daily during a period from 1 to 3 days. In two cases the strain developed resistance within 24 hours after the treatment began. Twenty of the patients with initially resistant diarrhoeal *Escherichia* strains had been subjected to streptomycin treatment before the strains were isolated. In those four cases where the susceptibility to streptomycin did not change, streptomycin had not been given.

In contrast to the findings of Ferguson *et al.* (1951), the strains that fermented salicin did not differ from the non-salicin fermenting strains in their susceptibility to streptomycin. All the *Escherichia* 111:B4:— and 55:B5:4 strains examined attacked salicin, but nevertheless almost without exception these types were resistant to streptomycin. Similarly, there were more streptomycin resistant strains among the salicin-positive *Escherichia* 111:B4:12 strains than among the salicin-negative strains. In eleven of the twelve cases in whom the strains became resistant to streptomycin, the strains were initially salicin-positive, but the ability to ferment salicin did not disappear.

EPIDEMIOLOGICAL AND CLINICAL ASPECTS OF ESCHERICHIA DIARRHOEA

Age and sex incidence. — When the diarrhoea group is divided at a certain age limit, it is found that the lower age limit, the greater is the relative proportion of *Escherichia* diarrhoea cases in the group below the age limit, i.e. the incidence of *Escherichia* diarrhoea increases as the ages of the patients decrease. By combining data of Tables 17, 18 and 19, it is observed, for example, that the percentage of *Escherichia* diarrhoea cases in the diarrhoea group over six months is 14.5±1.9 and in the group less than six months 24.3±1.9; for the age limit of 3 months, the respective percentages are 16.0±1.6 and 27.7±2.5. One-half of the 42 diarrhoeal premature babies in the present series were infected with diarrhoeal *Escherichia* types.

The incidence of the *Escherichia* 111:B4 type is very pronounced (15.0±2.2 %) in the group under three months; more than one-half of the cases positive for the 111:B4 sero-type belong to this age group (Table 17).

TABLE 17

CASES POSITIVE FOR ESCHERICHIA 111:B4 STRAINS IN VARIOUS AGE GROUPS IN THE DIARRHOEA GROUP AND IN THE GROUP COMPRISING NON-DIARRHOEAL PATIENTS AND HEALTHY INFANTS DURING THE PERIOD MARCH 1, 1950—OCT. 1, 1953

Age in months	Diarrhoeal cases			Non-diarrhoeal patients and healthy infants			Difference between percentages
	Total no. of cases	111:B4 positive		Total no. of cases	111:B4 positive		
		Number	%		Number	%	
< 1	84	21	25.0±4.7	497	9	1.8±0.6	23.2±4.7
1—2	223	25	11.2±2.1	416	1	0.2±0.2	11.0±2.1
3—5	192	13	6.8±1.8	323	3	0.9±0.5	5.8±1.9
6—12	255	17	6.7±1.6	663	4	0.6±0.3	6.1±1.6
13—24	90	4	4.4±2.1	195	3	1.5±0.9	2.9±2.3
Total	844	80	9.5±1.0	2094	20	1.0±0.2	8.5±1.0

TABLE 18

CASES POSITIVE FOR ESCHERICHIA 55:B5 STRAINS IN VARIOUS AGE GROUPS IN THE DIARRHOEA GROUP AND IN THE GROUP COMPRISING NON-DIARRHOEAL PATIENTS AND HEALTHY INFANTS DURING THE PERIOD JAN. 1, 1951—OCT. 1, 1953

Age in months	Diarrhoeal cases			Non-diarrhoeal patients and healthy infants			Difference between percentages
	Total no. of cases	55:B5 positive		Total no. of cases	55:B5 positive		
		Number	%		Number	%	
< 1	51	3	5.9±3.3	454	21	4.6±0.9	1.3±3.4
1—2	154	9	5.8±1.9	378	8	2.1±0.6	3.7±2.0
3—5	134	3	2.2±1.3	303	3	1.0±0.5	1.2±1.4
6—12	204	6	2.9±1.2	614	8	1.3±0.5	1.6±1.3
13—24	81	3	3.7±2.1	188	—	0 ±0.2	3.7±2.1
Total	624	24	3.8±0.8	1937	40	2.1±0.3	1.7±0.9

TABLE 19

CASES POSITIVE FOR ESCHERICHIA 26:B6 AND 44:K? STRAINS IN VARIOUS AGE GROUPS IN THE DIARRHOEA GROUP AND IN THE GROUP COMPRISING NON-DIARRHOEAL PATIENTS AND HEALTHY INFANTS DURING THE PERIOD MAY 2, 1951—OCT. 1, 1953

Age in months	Diarrhoeal cases					Non-diarrhoeal patients and healthy infants					Difference between percentages	
	Total no. of cases	26:B6 positive		44:K? positive		Total no. of cases	26:B6 positive		44:K? positive		26:B6 positive	44:K? positive
		Number	%	Number	%		Number	%	Number	%		
<1	46	4	8.7±4.2	6	13.0±5.0	412	3	0.7±0.4	9	2.2±0.7	8.0±4.2	10.8±5.1
1—2	139	12	8.6±2.4	6	4.3±1.7	354	4	1.1±0.6	2	0.6±0.6	7.5±2.4	3.7±1.8
3—5	121	17	14.1±3.2	4	3.3±1.6	275	2	0.7±0.5	2	0.7±0.5	13.4±3.2	2.6±1.7
6—12	180	15	8.3±2.2	3	1.7±1.0	575	9	1.6±0.5	4	0.7±0.3	6.7±2.2	1.0±1.0
13—24	78	1	1.3±1.3	1	1.3±1.3	183	—	0±0.2	1	0.5±0.5	1.3±1.4	0.8±1.4
Total	564	49	8.7±1.2	20	3.5±0.8	1799	18	1.0±0.2	18	1.0±0.2	7.7±1.2	2.5±0.8

The number (96) of diarrhoeal boys infected by diarrhoeal *Escherichia* types was larger than the number (76) of girls; the percentage for the former was 55.8±3.8 and for the latter 44.2±3.8.

Seasonal incidence. — When the cases were grouped according to the season, it was established that the incidence was highest in Summer. Seventy or 40.7±3.8 per cent of the 172 *Escherichia* diarrhoeal cases were diagnosed during the months of June, July and August, whereas the percentages for the periods September—November and March—May were 17.5±2.9 and 22.6±3.2. Owing to the period when the specimens were collected, specimens were taken during December—February during only three years; the percentage of *Escherichia* diarrhoea cases for this period in these years was 19.2±3.1.

The incidence of Escherichia types in different years. — The incidence of the various diarrhoeal *Escherichia* types and sub-types in the present series varied in different years. During the years 1950—51, the most frequently diagnosed diarrhoeal *Escherichia* type was 111:B4; the number of cases was 70. In 1952 nine and in 1953 only one case was observed. Cases infected with *E.coli* 55:B5 were most frequent (20 cases) in 1953, whereas only one case was diagnosed in 1951 and only three cases in 1952. Forty-four of the 49 *Escherichia* diarrhoeal cases found positive for *E.coli* 26:B6 were from 1952 and 1953.

A variation was also observed in the incidence of *Escherichia* strains with common O and K antigens when the H antigen was taken into account. A majority of the *E.coli* 111:B4 strains isolated in 1950 and in the beginning of 1951 were non-motile. From May 1951, all strains of this type possessed the H12 antigen. In this connection it may be mentioned that a strain with antigen H2 was isolated from the faecal specimens of an infant treated at the Oulu Provincial Hospital. Among the *E.coli* 55:B5 strains isolated in 1951 and 1952 there were, in addition to non-motile strains, strains which possessed antigen H6, H7 or H11. From June 1953, most of the diagnosed diarrhoeal *Escherichia* strains were of the 55:B5:4 sub-type.

Nosocomial infections. — At the Epidemic Hospital, where the infants are treated in cubicled wards, nosocomial infections associated with diarrhoeal *Escherichia* types are obviously uncommon. Only one of the 24 *Escherichia* diarrhoea cases treated in this hospital was observed to develop diarrhoea after having been treated for some time in the hospital; this patient was found negative for diarrhoeal *Escherichia* strains before the onset. Only one of the 113 controls from the same hospital was found positive for a diarrhoeal *Escherichia* strain and only after admission.

It was, however, seen in Table 7 that the number of cases found positive for diarrhoeal *Escherichia* strains after admission was very marked. This leads to the assumption that many of the *Escherichia* diarrhoea cases treated at the Children's Clinic were of nosocomial origin. The cases from the Clinic found positive for diarrhoeal *Escherichia* strains have therefore been classified according to the isolated *Escherichia* types by taking into account whether the strain has been isolated from specimens taken on or after admission (Table 20). Also those cases have been noted where diarrhoea had developed while the patient was in the hospital and no diarrhoeal *Escherichia* types had been isolated from the faeces before the onset of diarrhoea. From the table it is seen that 35 of the 60 established diarrhoea cases positive for *E.coli* 111:B4:—, 6 of the 18 established *E.coli* 111:B4:12 and 111:B4:? cases and 17 of the 40 *E.coli* 26:B6 cases developed diarrhoeal symptoms only after admission. The percentage of *Escherichia* diarrhoea cases who started an attack of diarrhoea in hospital was 44.1 ± 4.9, while 96.5 ± 2.0 per cent of non-diarrhoeal cases harbouring diarrhoeal *Escherichia* strains were found positive after admission. The large proportion of the *Escherichia* diarrhoea cases who were found positive for diarrhoeal *Escherichia* strains only after the development of diarroea symptoms in the hospital and the large number of controls also found positive for these strains after admission suggest that infection by diarrhoeal *Escherichia* strains occurred at the Children's Clinic.

The contamination of patients in hospital is shown by the following example. In June 1953, a diarrhoeal patient was admitted to the Children's Clinic who had been at the Helsinki Children's Castle one week earlier. An *E.coli* 55:B5:4 strain was isolated from a faecal specimen taken from this patient on admission. This

TABLE 20

CLASSIFICATION OF CASES ACCORDING TO WHETHER THEY WERE FOUND POSITIVE FOR DIARRHOEAL ESCHERICHIA TYPES ON OR AFTER ADMISSION TO THE CHILDREN'S CLINIC

The numbers in brackets give the cases with whom onset of diarrhoea occurred in the hospital

Diarrhoeal Escherichia type	Escherichia diarrhoea cases			Non-diarrhoeal cases positive for diarrhoeal Escherichia		
	Total no. of cases	Found positive		Total no. of cases	Found positive	
		On admission	After admission		On admission	After admission
111:B4:—	60	6	54 (35)	8		8
111:B4:12	15	5	10 (5)	10	1	9
111:B4:?	3		3 (1)	1		1
55:B5:4	13	2	11	39		39
55:B5:—						
55:B5:6						
55:B5:7	2	2		1		1
55:B5:11						
55:B5:?						
26:B6:—	29	16	13 (3)	14	1	13
26:B6:11	10	7	3 (1)	4		4
26:B6:?	1		1			
44:K?	13	4	9	14	1	13
Total*	144	42	102 (45)	87	3	84
%	100.0	29.2±3.8	70.8±3.8 (44.1±4.9)	100.0	3.5±2.0	96.5±2.0

* Cases from whom two different types were isolated are counted only once

type had not been previously diagnosed at our laboratory. During the next four months, this same type was isolated from 52 patients in the Children's Clinic. With the exception of two patients, who were found positive on admission, the strains were isolated from the patients only after they had been admitted to the wards.

A casual observation made during the course of this investigation suggested a factor that may be of significance for the elucidation of nosocomial infections. After an ordinary house-fly was allowed to walk over a lactose agar plate situated in the autopsy room, several *E.coli* 111:B4:— colonies were later found growing on the plate. It

thus seems that the flies present in the wards can be potential carriers of diarrhoeal *Escherichia* strains.

Regional incidence. — With the exception of the hospital itself, it was not possible to determine any locality from which a greater number of *Escherichia* diarrhoea cases had arrived at the hospital. Of the diarrhoeal patients found to be excerting diarrhoeal *Escherichia* strains on admission, 35 were from different areas of the City of Turku, whereas 22 of the patients were from rural areas near this city. One case positive for *E.coli* 111:B4:2 was from Oulu and three cases positive for *E.coli* 26:B6 from Pori.

Case-fatality rate. — In the Children's Clinic the fatality rate of infantile diarrhoea has decreased considerably since the end of the period 1939—1949, during which it was 17.2±0.9 per cent (Salmi and Grönroos 1951). In the present series deaths resulted from diarrhoea in 2.5±0.5 per cent of all diarrhoeal cases. The case-fatality rate among the diarrhoeal patients positive for diarrhoeal *Escherichia* types was 4.1±1.5 per cent. Of the seven fatal *Escherichia* diarrhoea cases four had been found to excrete *E.coli* 111:B4 strains, two *E.coli* 55:B5 strains and one *E.coli* 26:B6 strain; five of these were less than one month old, four premature babies.

Clinical features. — The symptoms associated with *Escherichia* diarrhoea did not differ essentially from those of infantile diarrhoea described in textbooks, which include vomiting, watery stools, loss of weight, dehydration and fever. Consequently *Escherichia* diarrhoea can be diagnosed only by bacteriological and serological methods.

As examples, two case reports of *Escherichia* diarrhoea patients are presented below. The onset of diarrhoea in the second case occurred in hospital.

Case 1. (Record No. 915/52) The child was a four months old daughter of a labourer. According to the patient's mother, the patient had suffered from diarrhoea during one week and had vomited profusely. No cases of diarrhoea had occurred in the neighbourhood of the child's home. As the child could not drink or eat because of the vomiting, she was brought to the hospital. The child's general condition was poor; she was tired, pale, drousy, dehydrated and feverish and passed watery

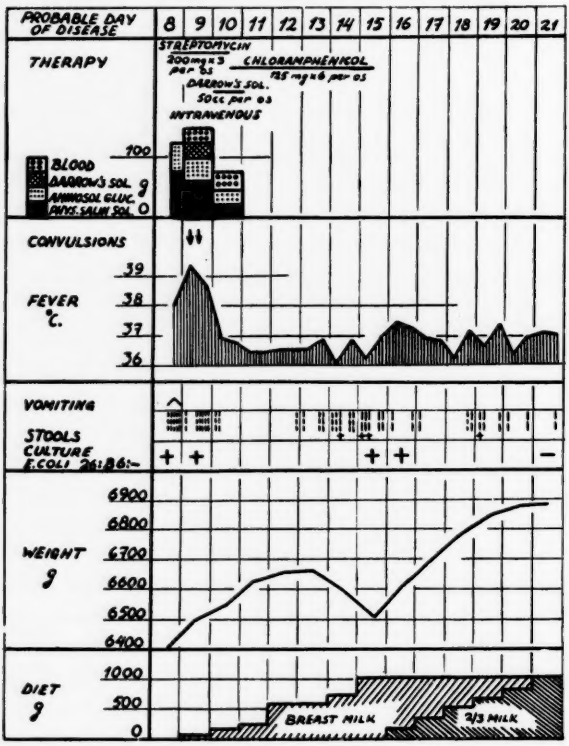

Fig. 1. Chart relating to case 1.

stools. *E.coli* 26:B6:— was isolated from the faecal specimen taken on admission. The child recovered rapidly after fluid replacement, antibiotic, and diet treatments. Even after the antibiotic therapy, the abovementioned *Escherichia* strain was still found in the faeces specimens which at that time were somewhat watery and slimy. The patients was discharged after two weeks in the hospital. No *Escherichia* strain was cultured from the faeces specimen taken on discharge nor from a specimen taken 12 months later.

Case 2. (Record No. 1338/52) The patient was a three months old son of a labourer, and had been brought to hospital because he suffered from a respiratory infection. The child was recovering from his cough when on his eighth day at the hospital he started to vomit, his weight decreased, and he passed several watery stools. No diarrhoeal *Escherichia* strains were isolated from faecal specimen taken on admission, but during the diarrhoea period, *E.coli* 111:B4:— was isolated. The child recovered rapidly after receiving an adequate diet and phthalazole therapy. When the patient was discharged a few days later, the diarrhoeal *Escherichia* strain could not be isolated from the faeces.

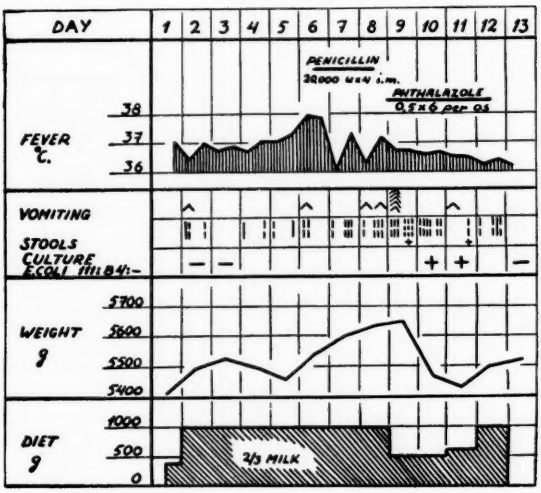

Fig. 2. Chart relating to case 2.

In general the *Escherichia* diarrhoea cases were mild. Of the cases in whom diarrhoea was associated with intoxication, 38.5±9.5 per cent were *Escherichia* diarrhoea cases. *Escherichia* diarrhoea cases appeared to be more frequent in the group of toxic cases than in the groups of diarrhoea patients with respiratory infections and without associated infections (Table 21).

TABLE 21

THE INCIDENCE OF ESCHERICHIA DIARRHOEA IN VARIOUS TYPES OF DIARRHOEA IN THE PRESENT SERIES

Type of disease	Diarrhoea	Escherichia diarrhoea	
	Total no. of cases	No. of cases	%
Gastrodyspepsia	23	6	26.1±9.2
Diarrhoea	586	119	20.1±1.7
Diarrhoea + intoxicatio	26	10	38.5±9.5
Diarrhoea + infectio ac. respirat. .	162	24	14.8±2.8
Diarrhoea + infectio ac. non respirat.	47	13	27.8±6.5
Total	844	172	20.2±1.4

Carriers. — Of the 258 hospitalized cases found positive for diarrhoeal *Escherichia* types, 157 or 60.9±3.4 per cent were found to be negative before their discharge from the hospital. Information relating to the remaining patients was not obtained.

In order to detect possible carriers of diarrhoeal *Escherichia* strains, three faecal specimens were requested from 125 discharged patients found positive for diarrhoeal *Escherichia* strains in hospital. Specimens were obtained from 56 of these and were taken at intervals of several days with the assistance of health nurses. This follow-up study was performed in 9 cases 1—3 months, in 6 cases 4—6 months, in 15 cases 7—12 months and in 26 cases more than one year after the first positive specimen was diagnosed. In none of these cases was the previously isolated diarrhoeal *Escherichia* strain found in the faeces.

III. DISCUSSION

In the series under study, *E.coli* types 111:B4, 26:B6 and 44:K? were more often isolated from the faeces of diarrhoeal children under two years of age than from the faeces of non-diarrhoeal and healthy infants of the same age. No significant difference is, however, noted in the frequency of *E.coli* 55:B5 strains between the two infant groups.

Only two of the 14 diarrhoeal patients positive for *E.coli* 55:B5:4 strains were found to excrete this strain on admission, whereas 8 of the 10 cases infected with other 55:B5 sub-types were found positive already on admission. The number among the controls who became infected with the *E.coli* 55:B5:4 sub-type in hospital was 39. Only one control case was found to harbour another sub-type than E.coli 55:B5:4. Even the small number of cases positive for other 55:B5 sub-types than 55:B5:4 may be construed to indicate some relationship between these sub-types and infantile diarrhoea. It should be noted that almost all of the control cases that were found positive for the 55:B5:4 sub-type had been given prophylactical oxytetracycline treatment, which may have possibly prevented the development of diarrhoea. The aetiological role of the 55:B5 sub-types in infantile diarrhoea thus remains unclear. It should be remembered, however, that 55:B5 strains have been frequently found to be associated with infantile diarrhoea. It thus remains for future investigation to determine to what extent 55:B5 and other diarrhoeal *Escherichia* types with different H antigens are associated with infantile diarrhoea and whether these strains differ in their pathogenicity. For this reason all diarrhoeal *Escherichia* strains isolated should be defined as to their H antigens.

The relatively large number of patients who were found negative for diarrhoeal *Escherichia* strains on admission and who developed

diarrhoea in hospital, whereupon diarrhoeal *Escherichia* strains were isolated at the acute stage, provide evidence of the aetiological significance of diarrhoeal *Escherichia* types in infantile diarrhoea.

The relative increases in the number of patients found positive for diarrhoeal Escherichia types during their stay in the hospital in the diarrhoea and non-diarrhoea groups reveal that the incidence of cases positive for diarrhoeal *Escherichia* types would probably have been much lower if the patients had not been exposed to contamination after their arrival at the hospital. It can safely be considered that the non-contact cases positive on admission give more reliable data about the incidence of diarrhoeal *Escherichia* strains outside the hospital. The incidence is about thirty times greater in the diarrhoeal group (6.5±0.9 %) than in the control group (0.2±0.1 %). This also supports the assumption that these types are of aetiological significance in infantile diarrhoea.

As the *Escherichia* strains isolated from 1133 pus and urine specimens did not belong to the diarrhoeal *Escherichia* types and as diarrhoeal types were in only few cases isolated from non-contact controls, it seems that diarrhoeal *Escherichia* strains show a marked specificity in that they are frequently present in infantile diarrhoea but obviously very rarely in non-diarrhoeal conditions.

During the period of investigation, it became evident that *Salmonellae* and *Shigellae* have only a minor importance as causative organisms in infantile diarrhoea, which is in accord with many previous studies conducted elsewhere. Infants from whom diarrhoeal *Escherichia* types were isolated comprised 20.2±1.4 per cent of all diarrhoea cases in the series, which is a much higher proportion than that attributable to *Salmonella* infections, which amounted to only 2.4±0.5 per cent.

Each diarrhoeal *Escherichia* sero-type appears to possess its own fermentation reactions to a large degree. With respect to some reactions, variation may be encountered, but even then some regularity may noted as, for instance, in the case of 111:B4 types, which ferment sorbose late, only after an incubation period of 20 days, or even fail to attack it. Consequently, although it is not possible to draw conclusions about the O and K antigens of a strain on the basis of its fermentation reactions, it is, however, possible to do this in some cases with respect to H antigens within

a certain OK group. Thus, for example, inability of an *E.coli* 111:B4 strain to ferment sucrose points to the presence of the H 12 antigen.

Antibodies against diarrhoeal *Escherichia* strains were detected in sera of patients to only a limited extent. Sera in which bacterial agglutinins were established were few in number. In only a few cases were *E.coli* O111, O55 and O26 haemagglutinins found present with higher titres in sera of patients positive for the homologous strains than in the sera of the controls. Further investigations on a greater number of cases are therefore necessary to clarify the significance of the indirect bacterial haemagglutination test in infantile *Escherichia* diarrhoea.

The results of the studies on the drug susceptibilities of the diarrhoeal *Escherichia* types show conclusively that streptomycin therapy cannot be relied upon to give favourable results in the treatment of *Escherichia* diarrhoea; furthermore *Escherichia* strains soon acquire a resistance to this drug. This makes it easy to understand the discordant results obtained by different investigators who have studied the value of streptomycin therapy in infantile diarrhoea, but who have not followed the variation of the resistance to the drug. Although *in vitro* determinations cannot be expected to give reliable indications of the value of therapeutic agents in the treatment of individual cases, the results of the present investigation suggest that the most promising agent for the treatment of *Escherichia* diarrhoea is neomycin, which has not generally been used except for local therapy, especially since resistance to this drug develops much more slowly than resistance to streptomycin (Gorzynski and Neter 1953).

The spread of diarrhoeal *Escherichia* types in the hospital considered by several investigators (Smith *et al.* 1950, Braun and Henekel 1951, Laurell *et al.* 1951, Rogers and Koegler 1951, Laurell 1952 *a*, Schmidt *et al.* 1952, Müller 1953) appears to apply also in the present series in view of the large number of positive cases diagnosed after the patients had been in the hospital for some time. It has become evident that the possibilities for the spread of nosocomial diarrhoeal *Escherichia* infections are very great in our Children's Clinic. With the exception of the infants from the Child Welfare Centres and those treated at the Epidemic Hospital, the greater part of the children in the control group may

have been exposed to infection by diarrhoeal *Escherichia* strains. At the Children's Clinic, 44.1±4.9 per cent of the *Escherichia* diarrhoea cases developed diarrhoea in the hospital and 96.5±2.0 per cent of the patients of the control group positive for diarrhoeal *Escherichia* strains were found to harbour the strains only after they had been in the hospital for some time. The wards of the Children's Clinic, which were not originally intended to be used for the purpose, do not contain cubicles where diarrhoeal children may be isolated. The isolation of diarrhoeal *Escherichia* from faecal specimens from members of the nursing staff suggest that the nursing staff may act as carriers and promote the spread of infection in the hospital.

From the epidemiological viewpoint, the determination of the H antigen component is of pronounced value for the detection of nosocomial infections and possibly also for the elucidation of the relationship between strains of the same OK type and infantile diarrhoea. The importance of this has been previously stressed also by Wright (1953) and Wright *et al.* (1953). Another step which may be of value is the determination of fermentation types, although this is of lesser significance than the preceding. With only a few exceptions, strains with the same antigenic structure isolated from the same outbreak have been of the same fermentation type.

The symptoms of diarrhoea associated with diarrhoeal *Escherichia* types do not differ significantly from those of infantile diarrhoea in general. The diagnosis of *Escherichia* diarrhoea can hence be made only by isolating a diarrhoeal *Escherichia* strain and conducting a serological type determination. The author's opinion is that this should be done in all suspected cases of infantile diarrhoea, at least when such cases are admitted to hospital. Routine specimens should be taken for the diagnosis of *Escherichia* diarrhoea as is the case generally for typhoid and paratyphoid fevers.

IV. SUMMARY AND CONCLUSIONS

The purpose of the investigation has been to collect additional information on the possible aetiological significance of certain *Escherichia coli* sero-types in diarrhoea in the age group under two years, to determine the serological and bacteriological properties of the isolated *Escherichia* types and to pay attention to epidemiological and clinical aspects of infantile diarrhoea in the light of these findings.

In a review of the earlier papers on this subject, also the recent work on the serological and bacteriological properties of *Escherichia* types, particularly of those associated with diarrhoea, has been outlined.

The *Escherichia* types designated as diarrhoeal *Escherichia* types in this investigation are the sero-types 111:B4: 55:B5, 26:B6, 44:K?, 86:B7, E 611 and Canioni. Cases of diarrhoea from which one of these sero-types has been isolated have been referred to as *Escherichia* diarrhoea cases.

A total of 12045 faecal specimens and 6527 nose and throat specimens have been examined for the presence of diarrhoeal *Escherichia* types. The findings relate to 3754 patients, healthy children and members of nursing staffs. Among these subjects there were 2959 children under two years of age, of whom 865 had diarrhoea. The remaining 2094 children were non-diarrhoeal patients and healthy infants; this group has been considered a control group. These latter two groups have been subjected to a more detailed analysis.

The serological and bacteriological properties of 907 diarrhoeal Escherichia strains from 448 faecal cultures have been investigated.

One hundred and seventy-two sera from 155 patients have been examined to detect agglutinins against the diarrhoeal *Escherichia*

types. One hundred and sixty-five serum specimens from 154 patients have been examined for the presence of *Escherichia* O111, O55 and O26 haemagglutinins. Ninety-eight of the former patients and 44 of the latter were positive for diarrhoeal *Escherichia* strains.

Drug susceptibilities of the diarrhoeal *Escherichia* strains have been studied *in vitro* using the following seven therapeutic agents: streptomycin, chloramphenicol, chlortetracycline, oxytetracycline, polymyxin B, neomycin and sulphathiazole.

Attention has been paid to the incidence of *Escherichia* diarrhoea in different infants age groups and to the frequency of different *Escherichia* types. The occurrence of nosocomial infections associated with diarrhoeal *Escherichia* types has been considered and certain observations on the nature of *Escherichia* diarrhoea infections, on the case-fatality rate and on carriers have been presented.

The following conclusions may be drawn on the basis of the findings of the investigation.

1. The *Escherichia* sero-types 111:B4, 26:B6 and 44:K? appear with a higher frequency in the faeces of diarrhoeal children under two years of age than in those of non-diarrhoeal and healthy children of the same age. Of the diarrhoeal cases from whom diarrhoeal *Escherichia* types were isolated, 9.5± 1.0 per cent were found to harbour the sero-type 111:B4 and 8.7±1.2 per cent the sero-type 26:B6. In the control group, the respective percentages were both 1.0±0.2. *E.coli* 55:B5 strains were found in 3.8±0.8 per cent and 44:K? strains in 3.5±0.8 per cent of the diarrhoeal infants; in the control group, the percentages were 2.1±0.3 and 1.0±0.2. Only one diarrhoea case was found which harboured a *E.coli* E 611 strain.. *E.coli* 86:B7 and Canioni type strains were not isolated. In summary, 20.2±1.4 per cent of the diarrhoea cases were found to be *Escherichia* diarrhoea cases, whereas 4.4±0.5 per cent of the control group were bearers of diarrhoeal *Escherichia* strains.

   During the period of investigation, *E.coli* 111:B4 and 26:B6 were thus the types most frequently encountered in the

diarrhoea cases. It may thus be considered that these two sero-types are closely associated with infantile diarrhoea.

The significance of the *E.coli* 55:B5 sub-types remains unclear. Further investigations are therefore necessary to determine to what extent these sub-types are associated with infantile diarrhoea.

2. The following diarrhoeal *Escherichia* sero-types were identified: 111:B4:—, 111:B4:2, 111:B4:12, 55:B5:—, 55:B5:4, 55:B5:6, 55:B5:7, 55:B5:11, 26:B6:—, 26:B6:11, 44:K?:— and 44:K?:18. In addition, 4 isolated strains of 111:B4, 14 of 55:B5, 6 of 26:B6 and 15 of 44:K? type were found to be motile, but the determination of their H antigens did not succeed with the H immune sera 1—33. The flagellar antigen of the isolated E 611 strain was found to be H 2.

The sub-type *E.coli* 55:B5:4 is a new type in respect of its H antigen and has not been previously described. Neither have the *E.coli* 44:K? sub-types been described earlier.

One hundred and forty-three of the 144 isolated *E.coli* 26:B6:— strains and 14 of the 15 isolated *E. coli* 44:K?:? strains were found to be capable of lysing sheep red cells within 24 hours. None of the other strains was haemolytic.

Diarrhoeal *Escherichia* agglutinins were detected in a few cases, but to such a limited extent that these findings have no practical significance.

Drug susceptibility tests conducted *in vitro* revealed that a large proportion of the diarrhoeal *Escherichia* strains (63.8 ±2.3 %) were resistant to streptomycin. A concentration of 20 mg per cent sulphathiazole was not found to prevent the growth of 33.8±2.9 per cent of the diarrhoeal *Escherichia* strains. The strains were, however, sensitive to chloramphenicol, chlortetracycline, oxytetracycline, neomycin and polymyxin B.

3. Diarrhoeal *Escherichia* strains were present in a higher proportion of the diarrhoeal infants less than six months old (24.3±1.9 %) than in the older infants (14.5±1.9 %). The high incidence (15.0±2.2 %) of the type 111:B4 in diarrhoeal infants less than three months old is striking.

Diarrhoeal *Escherichia* cases were found to be more prevalent during the Summer months, June to August

(40.6±3.8 %). The incidence of the diarrhoeal *Escherichia* types varies in different years.

The determination of the H antigens and the study of the fermentation reactions may be of decisive value in the elucidation of the epidemiology of *Escherichia* diarrhoea.

The large proportion (44.1±4.9 %) of the cases who developed diarrohea in hospital and who were then found positive for diarrhoeal *Escherichia* strains and the large number (96.5±2.0 %) of the subjects of the control group who were initially negative but were later found positive for diarrhoeal *Escherichia* strains suggest that measures should be taken to prevent the spread of infections associated with these organisms within the hospital.

The fatality rate of *Escherichia* diarrhoea during the period of investigation was 4.1±0.5 per cent.

No carriers were detected among 56 discharged infants earlier found positive for diarrhoeal *Escherichia* strains.

The conclusions made suggest the following practical measures. Faecal specimens should be taken from every infant admitted to a hospital and cultured to determine the presence of diarrhoeal *Escherichia* strains. In order to avoid nosocomial infections, a child found positive for diarrhoeal *Escherichia* types should be isolated as in the case of other contagious diseases; especially this should be done for all diarrhoeal patients that are found to harbour diarrhoeal *Escherichia* strains, as is usual in suspected cases of *Salmonella* infections.

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ERRATA

P. 5, line 13: *for* Wikström *read* Wickström
P. 6, line 12: *for* Mr. Korte *read* Mr. R. Korte
P. 13, line 34: *for* (1952) *read* (1952, 1953)
P. 14, line 31: *insert* Kröger and Dölle 1953,
P. 16, line 3: *for* 86:B7 (Ørskov 1951) *read* 86:B7 (Ørskov 1954)
P. 19, line 3 from bottom: *for* agent *read* agents
P. 23, line 2: *for* bacteria *read* bacterial
P. 24, line 2 from bottom: *for* plates *read* plate
P. 26, line 27: *for* Wahlne *read* Vahlne
P. 28, line 25: *for* Coomb's *read* Coombs
P. 39, Table 9, col.2: *for* Bi 7453/41 *read* Bi 7458/41
P. 40, line 8: *for* by one *Escherichia* 26:B6: — *read* by one *Escherichia* 111:B4:? and one 26:B6: —
P. 41, line 16: *for* 55:B5:6 *read* 55:B5:4
P. 44, line 2 from bottom: *for* sera taken *read* sera were taken
P. 45, line 7 from bottom: *for* which *read* who
P. 50, Table 18, col. 7: *for* 0 ± 0.2 *read* 0 ± 0.5
P. 51, Table 19, col. 9: *for* 0 ± 0.2 *read* 0 ± 0.5
P. 53, line 23: *for* 17 *read* 4
P. 55, line 4: *for* from which *read* where
line 5: *for* arrived at the hospital *read* developed
line 6: *for* excerting *read* excreting
P. 56, line 6: *for* No *Escherichia read* No diarrhoeal *Escherichia*
P. 60, line 6 from bottom: *for* may noted *read* may be noted
P. 62, line 10: *for* suggest *read* suggests
P. 68, line 17: *for* 1219 *read* 219
line 3 from bottom: *for* Zbl.Bakt.Orig. *read* Ztschr. Immunitätsforsch.
P. 70, line 1: *for* Ibid *read* Monatsschr. Kinderh.
line 11: *for* Ibid *read* Proc.Soc. Exper. Biol. & Med.